SCIENTIFIC PROCEEDINGS.

VOL. XXV. MARCH, 1928. No. 6.

Western New York Branch.

University of Buffalo Medical School, February 11, 1928.

3868

Vasoconstriction in the Liver.

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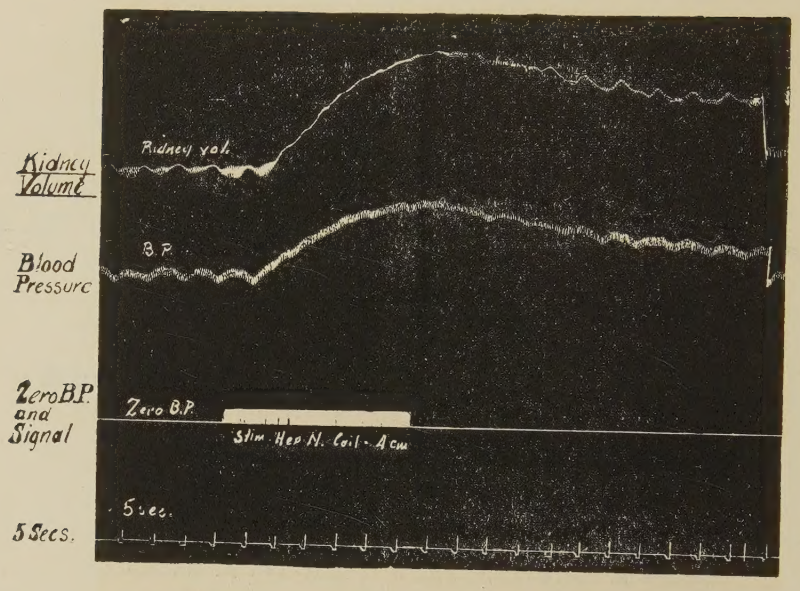
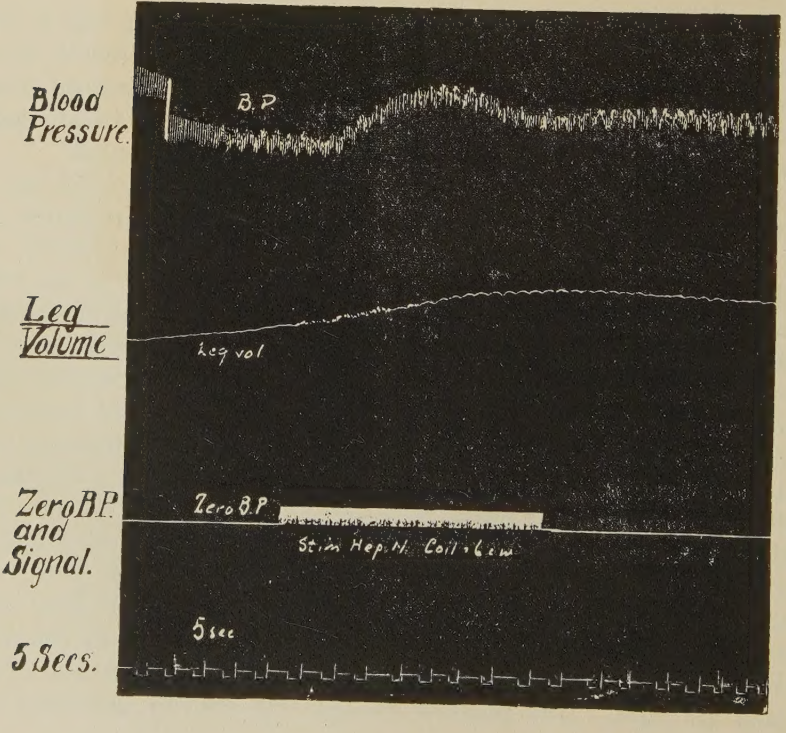
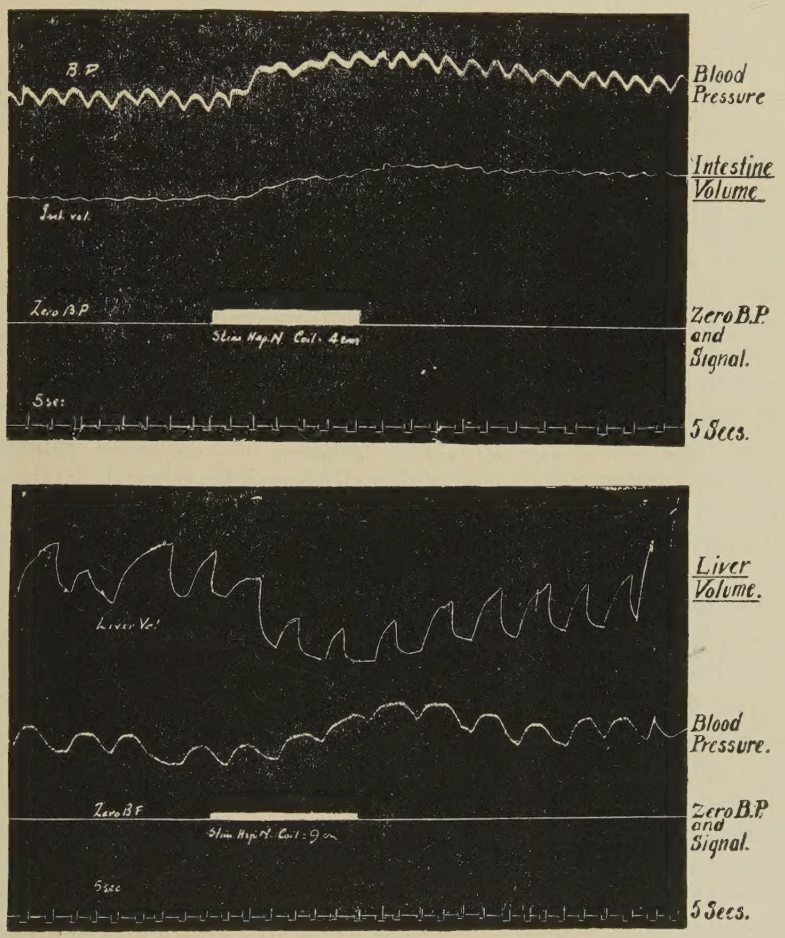
Stimulation of the nerves going to the liver causes a rise in the arterial blood pressure in the cat.[1] It has been attempted to show that this is due to the release of a vaso-constrictor substance from the liver into the general circulation; but the effort to isolate such a substance from extracts of the liver has been unsuccessful.[2]

We have recently taken records of the volume of a hind leg, a kidney, an intestinal loop and the liver itself during stimulation of its nerves. The experiments were done on cats under urethane or chloralose anesthesia. Shielded electrodes were applied to the hepatic nerves after they had been dissected free from the artery proximal to its gastro-duodenal branch; the nerves were ligated and cut centrally to the electrodes and were stimulated with a tetanizing current from a Harvard inductorium. The leg, kidney and intestinal volumes were recorded in the usual way. A special plethysmograph was devised for use with the liver. This was, essentially, a flattened, bell-shaped device that could be lowered over the entire liver—the animal being on its back and the liver exposed by a ventral, mid-line incision. A notch on each side of the plethysmograph prevented occlusion of the arterial or venous (portal and hepatic) blood vessels; and the lower edges were conformed to the shape of the dorsal body wall so that when snugly in place the junction was practically air tight. Any possible leakage was entirely prevented by


Kidney Volume
Kidney vol.
Blood Pressure
B.P.
Zero B.P. and Signal
Zero B.P.
Stim. Hep. N. Coil - 4 cm
5 Secs.
5 sec.


Blood Pressure
B.P.
Leg Volume
Leg vol
Zero B.P. and Signal.
Zero B.P.
Stim Hep. N. Coil - 6 cm
5 Secs.
5 sec



pouring enough paraffine oil into the abdominal cavity to cover the lower edges.

The records, of which examples are reproduced in the accompanying figures, show that the rise of arterial blood pressure which results from stimulation of the hepatic nerves is accompanied by, or results in a passive dilatation of the leg, kidney and intestines; the liver alone decreases in volume. Such evidence would seem to indicate that the rise in blood pressure is due merely to the forcing of blood from the extensive vascular bed in the liver into the general circulation rather than to the release of a vascoconstrictor substance from the liver into the blood stream.

[1] Cannon, W. B., and Uridil, J. E., *Am. J. Physiol.*, 1921, lviii, 353.

[2] Cannon, W. B., and Griffith, F. R., *ibid.*, 1922, lx, 544.

3869

Relation Between Absorption and Utilization of Galactose.

CARL F. CORI AND GERTY T. CORI.

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Previous experiments[1] have shown that when galactose and glucose are absorbed together, the rate of absorption of galactose is greatly reduced. Folin and Berglund[2] had reported previously that in men the sugar excretion is less than one-tenth as great when a mixture of glucose and galactose (100 gm. each) is ingested as the excretion obtained from 100 gm. of galactose when taken alone. They suggested that the extent to which galactose is utilized in the human organism depends on the quantity of available glucose. Corley[3] administered glucose and galactose intravenously and obtained no evidence that the presence of an excess of glucose in the blood increased the ability of the rabbit to utilize circulating galactose. However, when Corley[4] administered glucose and galactose by mouth, the urinary excretion of galactose decreased, in confirmation of the results of Folin and Berglund. These observations made it desirable to establish a more definite relationship between the rate of absorption and the extent of utilization of galactose in the body. Such experiments were made 2 years ago and are now here reported.

Experimental. Each group, consisting of 4 to 6 rats, fasted previously for 48 hours, was fed a different amount of galactose by stomach tube, the amount introduced being known in each case. In one series of experiments galactose alone was fed, in a second series a galactose-glucose mixture of equal parts was given, and in a third series, galactose was presented to the rats in the form of lactose. The collection of urine was extended beyond the period of absorption in order to make allowance for any lag in sugar excretion. Figure 1 is a graphic illustration of the average values obtained in these experiments. The absolute amounts as well as the percentages of excretion and utilization corresponding to the varying amounts of galactose absorbed can be calculated from this graph. It will be noticed that in each of the 3 series of experiments the excretion of galactose, when plotted against the amount absorbed, falls on a straight line. The somewhat lower excretion in the beginning of absorption, represented by a slight break in the straight lines, is probably due to the establishment of the initial equilibrium between the galactose concentration of the blood and of the tissues. The re-

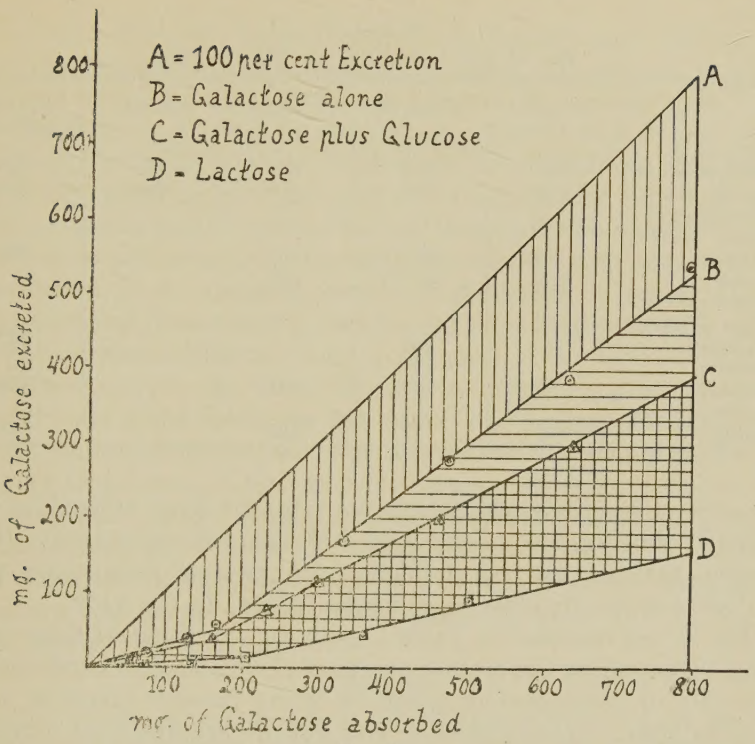

FIG. 1.

Excretion and utilization of galactose, when galactose alone, galactose plus glucose, and lactose is fed.

lation between excretion and utilization is indicated by the shaded areas. The vertically shaded area between lines A and B serves as an index of the amount of absorbed galactose which is utilized in the body, when galactose alone is fed, while the area included between B and the abscissa corresponds to the amount excreted. The area between A and C represents utilization of galactose, when a galactose-glucose mixture is fed and the horizontally shaded area between B and C corresponds to the increment in utilization (or decrease in excretion) with a galactose-glucose mixture as compared with galactose alone. What is the explanation for the phenomenon that for an equal amount of galactose absorbed, the utilization of galactose is lowest when galactose is being absorbed alone, intermediate when a galactose-glucose mixture is fed, and highest when lactose is ingested? We believe that this phenomenon is explained by different rates of absorption of galactose in these 3 cases. In Table I the ratio in the rate of absorption of galactose alone, or galactose from a galactose-glucose mixture and of galactose from lactose is of

TABLE I.
Mg. of sugar absorbed per 100 gm. rat.

	1 hour	2 hours	3 hours	4 hours	Average blood sugar
Galactose alone[7]	184	358	534	744	328
Glucose alone[7]	183	376	528	704	176
Galactose-glucose mixture					
Galactose	60	148(1)	264		220
Glucose	114	216(1)	309		
Galactose-fructose mixture					
Galactose		256			247
Fructose		182			
Lactose	56	104	162		128
Lactose from milk	48	92	129		118

the order 100:41:15, while the ratio in the amounts utilized, as calculated from Fig. 1, is of the order 100:153:240, respectively. It is obvious that the rate of absorption of galactose is inversely proportional to the amounts of galactose utilized in the body.

The values recorded in Table I, with the exception of those indicated by quotation figures, are averages of unpublished experiments. The rate of absorption of galactose alone remains constant from hour to hour. When galactose and glucose are absorbed together, the rate of absorption of galactose increases each hour, while the rate of absorption of glucose diminishes. This is due to the fact that glucose is absorbed faster from the mixture than galactose. Consequently, the molecular ratio between the 2 sugars in the intestine changes in favor of galactose as the absorption proceeds. From a galactose-fructose mixture the former sugar is absorbed faster than the latter. This is of interest in view of the results recorded by Bodansky,[5] who found that fructose in contradistinction to glucose, had only a slight effect on galactosuria. The low rate of absorption of lactose as compared with the rate of absorption of the galactose-glucose mixture must be due to the fact that the disaccharide is split rather slowly into its components by the intestinal lactase and that the disaccharide as such does not seem to be absorbed from the intestine of the rat. It is of interest that the absorption of lactose from milk proceeds at a still slower rate and that under these conditions practically no galactose is excreted in the urine. The lower rate of absorption from milk is probably due to the simultaneous absorption of amino acids.[6]

The blood sugar concentrations in Table I reflect the varying rates of absorption of galactose under the different conditions. The

blood sugar values in Table I are for total blood sugar, that is, for galactose plus glucose plus residual reduction. It seemed of interest to determine galactose and glucose separately in order to find out whether there was a rise in the glucose concentration of the blood during galactose absorption. Such a rise would indicate that galactose is converted in part into glucose. Considerable time has been spent in working out a method for the determination of glucose and galactose in the same blood sample. The method consists in the determination of the reducing power of the blood by means of the Hagedorn and Jensen method before and after treatment with yeast. In the meantime a method based on the same principle has been published by Corley,[8] who found that the recovery of added galactose ranged from 90 to 104%, the low results having been obtained with small amounts of galactose. Our recoveries were somewhat better, ranging from 96 to 102%, probably because the ferricyanide of the Hagedorn and Jensen method is more sensitive to small amounts of galactose than the Shaffer-Hartmann reagent. Table II indicates that during the absorption of galactose there is no rise in the glucose concentration of the blood. The same conclusion can be drawn from Corley's experiments.

TABLE II.

Galactose and glucose content of blood during galactose absorption (in mg. %).

	0 hours	1 hour	2 hours	3 hours
Rats				
Galactose	—	233	256	268
Glucose*	97	96	81	101
Cat				
Galactose	—	84	45	39
Glucose*	78	87	91	89
Rabbit				
Galactose	—	99	172	134
Glucose*	96	91	87	102

*Glucose plus residual reduction.

Summary. For an equal amount of galactose absorbed, the utilization is highest with lactose, intermediate with a galactose-glucose mixture, and lowest with galactose. This is ascribed to the different rate of absorption of galactose in the 3 cases, the rate being lowest with lactose, intermediate with a galactose-glucose mixture and highest with galactose. During the absorption of galactose there is no rise in the glucose content of the blood.

[1] Cori, C. F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 290.

[2] Folin, O., and Berglund, H., *J. Biol. Chem.*, 1922, li, 213.

[3] Corley, R. C., *J. Biol. Chem.*, 1927, lxxiv 19.

[4] Corley, R. C., *J. Biol. Chem.*, 1928, lxxvi, 23.
[5] Bodansky, M., *J. Biol. Chem.*, 1923, lvi, 387.
[6] Cori, C. F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 125.
[7] Cori, C. F., *J. Biol. Chem.*, 1926, lxx, 577.
[8] Corley, R. C., *J. Biol. Chem.*, 1927, lxxiv, 1.

3870

The Rate of Excretion of Galactose.

CARL F. CORI AND GERTY T. CORI.

From the State Institute for the Study of Malignant Disease, Buffalo.

It has been shown in a previous paper[1] that the percentage of absorbed galactose that is excreted in the urine of the rat increases with increasing length of absorption, in spite of the fact that the rate of absorption remains constant from hour to hour. Thus in 1

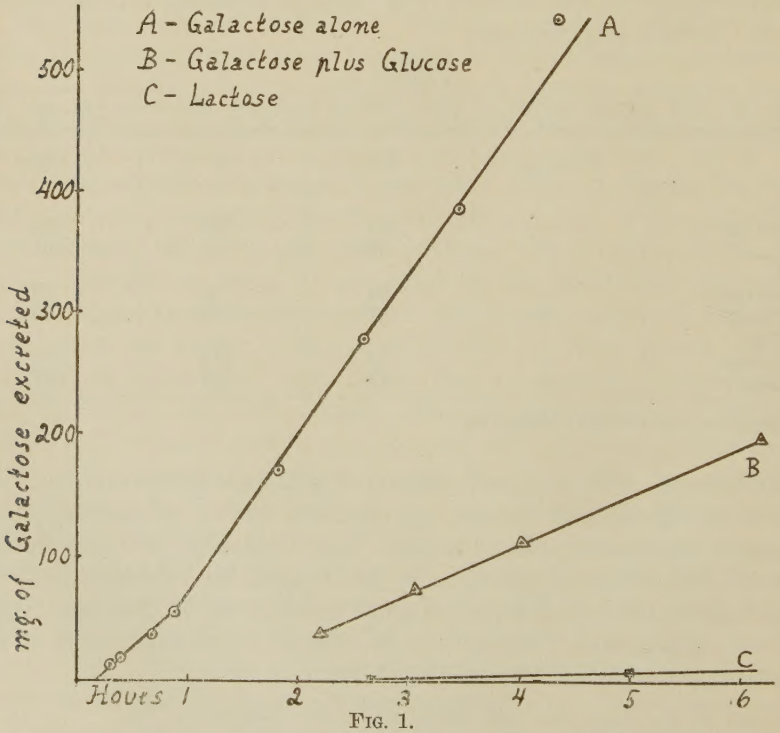

FIG. 1.

Rate of excretion of galactose, when galactose alone, galactose plus glucose, and lactose is fed.

TABLE I.
Galactose absorbed, utilized and excreted (in mg. per 100 gm. rat).

Galactose alone	1 hour	2 hours	3 hours	4 hours
Absorbed	182	364	546	728
Utilized	112	164	216	268
Excreted	70(50)	200(148)	330(279)	460(440)
% excreted	38.4(27.4)	55.0(41.0)	60.5(51.0)	63.2(60.5)

hour 27.4% appeared in the urine, in 2 hours 41%, in 3 hours 51%, and in 4 hours 60.5%. The present report is an attempt to elucidate the basis of this phenomenon.

Experimental. In figure 1 of the preceding paper the amounts of galactose excreted in the urine have been plotted against the amounts absorbed. The amounts excreted, as shown in this figure, can be plotted against time with the aid of the values recorded in Table I of the preceding paper, using 182 mg. per hour for the average rate of absorption of galactose alone, 74 mg. per hour for galactose from the galactose-glucose mixture, and 27 mg. per hour for galactose from lactose. This has been done in Fig. 1 of the present report. From Fig. 1 the values for excretion in Table I have been read off. It should be emphasized that these values are for an ideal excretion, since they have been obtained under experimental conditions which exclude a lag in sugar excretion. The values in parentheses, which are taken from a previous paper,[1] indicate how much galactose can actually be excreted during each hour. It will be noted that the percentage of absorbed galactose that is excreted in the urine increases from hour to hour. This makes it appear as if there were an hourly increment in the rate of excretion, and conversely an hourly decrement in the rate of utilization, but the phenomenon is actually due to a lower rate of excretion in the first hour, represented by a break in the straight line in Fig. 1. If one calculates the values from 0 to 1 hour, from 1 to 2 hours, and so on, one finds that after the first hour 52 mg. are utilized, and 130 mg. (or 71.4%) are excreted per hour for each consecutive hour. In other words the rate of excretion and utilization remains entirely constant after the first hour. The same consideration applies to the galactose-glucose mixture, except that the excretion and utilization do not attain a constant rate until the second hour. The values per hour after the second hour for galactose from the galactose-glucose mixture are 34 mg. utilized, and 40 mg. (or 57.1%) excreted. The lower rate of excretion in the first hour is due to the initial penetration of galactose into the tissues. The rate of excretion does not become con-

stant until the galactose concentration in the tissues and the blood has risen to a certain definite level. On account of differences in the rate of absorption this level is reached sooner and is higher when galactose is being absorbed alone than if galactose is being absorbed from the galactose-glucose mixture. These differences in the rate of absorption have an influence on the amount of galactose utilized per hour. The ratio in the rate of absorption is of the order 100:41. Of galactose when absorbed alone 52 mg. are utilized per hour; of galactose from the galactose-glucose mixture 34 mg. are utilized per hour. This bears out the fact already established for glucose (Woodyatt, Sansum and Wilder[2]) that for an increment in the rate of supply of sugar only a portion of the extra amount supplied is lost in the urine. In other words, the amount of galactose utilized per unit of time increases with increasing rates of absorption.

Summary. After the establishment of an initial equilibrium, due to the penetration of galactose into the tissues, the rate of excretion and utilization of galactose remains constant from hour to hour. The amount of galactose utilized per unit of time increases with increasing rates of absorption.

[1] Cori, C. F., *J. Biol. Chem.*, 1926, lxx, 577.

[2] Woodyatt, R. T., Sansum, W. D., and Wilder, R. M., *J. Am. Med. Assn.*, 1915, lxv, 2067.

3871

Residual Reduction in Blood Filtrate After Treatment with Colon Bacillus.

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From the Clifton Springs Sanitarium and Clinic, Clifton Springs, New York.

To 5 cc. portions of Folin-Wu filtrate were added 10 drops of a solution containing 6.4% NaH_2PO_4 and 17.9% Na_2HPO_4. These were then inoculated with a strain of an organism culturally and morphologically typical of *Bacillus coli communis*. After 24 to 48 hours incubation the residual reduction was determined by the technique of Folin and Wu[1] modified as follows: Several dilute standards ranging from an equivalent of 0 to 0.020% in terms of the original blood were treated in the same manner as, and simultaneously with, the unknown solution. The color of the latter was then compared directly without dilution and without the aid of a colori-

meter with the color given by the standards. The readings of about 100 determinations on whole blood ranged from slightly below 0.010 to somewhat over 0.020%, with the greater part of the figures lying between 0.015 and 0.020%. Plasma values were one-third to one-fifth of those obtained without removing the cells. If varying amounts of solution were used together with sufficient water to make the volume 2 cc., the results were proportional to the amount of filtrate present. When glucose was added to such incubated material immediately before the analysis the sum of the added glucose and the residual reducing power was found by the standard technique of Folin and Wu.[2] If glucose was added and incubation continued the added glucose was destroyed in 12 to 18 hours. If the incubation of such filtrates was continued for several days there was sometimes a very slight further decrease in reducing power which may have been associated with contamination, as it was not observed when the solution was autoclaved before inoculation. In solutions of pure glucose similarly treated the reducing power decreased much more slowly and the opacity indicated the presence of much fewer organisms. Such solutions, if the glucose content was not too high, showed, after about 2 days, a practically complete absence of reducing power by the method used. The residual reducing compounds were not present in the organisms themselves, for centrifuged and uncentrifuged specimens gave values which appeared identical. Specimens filtered through washed and unwashed filter paper[2] also gave identical values. Blood and plasma obtained with precautions to insure sterility gave results similar to those obtained on the filtrates.

In about 50 experiments a similar technique based on the copper reduction method of Benedict[3] was used, generally on specimens which were simultaneously analyzed as described above. This method is not well suited for the determination because of the high color given by the blank. In spite of the difficulty in making the readings there was no doubt that it showed much less reducing substance present than did the technique of Folin and Wu, and in most determinations the color given by the unknown was indistinguishable from that of the blank. There was no perceptible difference between the color given by plasma and whole blood even in specimens where the difference by the Folin and Wu method was striking. Furthermore, when a known amount of glucose was added, the added glucose and no more was recovered. As an additional check a known amount of glucose was added to a pure glucose solution in which colon bacillus had been growing for some time, and in which the reducing power was greatly decreased. Here the total glucose

recovered was equivalent to the sum of the added glucose plus the residual reduction determined by the Folin-Wu technique.

The authors feel that these experiments show the presence in whole blood of reducing compounds which are not affected by colon bacilli. These compounds are present in much lower concentration in plasma, and are not included, or included only to a very slight extent, in the determination by Benedict's copper method.

[1] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81.

[2] Some of the control experiments were suggested in a private communication by Dr. Stanley R. Benedict.

[3] Benedict, S. R., *J. Biol. Chem.*, 1925, lxiv, 207.

3872

Lipoid Nephrosis in Adrenalectomized Rats and Guinea Pigs.

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From the Laboratories of the Buffalo General Hospital, the State Institute for the Study of Malignant Diseases and the Department of Physiology of the University of Buffalo.

A paper recently published[1] describes lipoid nephrosis in completely adrenalectomized cats. We wished to know whether a similar condition developed in guinea pigs which, judging from the relative size of the adrenals to body weight, make greater use of the adrenals than any other animal, and in rats, which are known to require the adrenals less than most other mammals.

The guinea pig adrenals were destroyed in 2 stages 1 week apart, by electric cautery, care being taken to injure no other organs. The rat adrenals were removed through a single slit in the skin in the mid-line of the back by the lumbar path at one operation.

It was shown by autopsy that the guinea pig adrenals had been completely destroyed. The average survival, exclusive of 1 animal which died from overheating and 2 which died from shock, was 8.8 days. Although 7 animals are an insufficient basis to draw up a conclusive average for the survival period, the results would seem to indicate that there is little difference from cats.

The rats shown in the table belong to a series of 60 operated animals. Since only those animals which were in poor condition were turned over for histological examination, the periods of survival shown in the table are not representative of the whole series. The

accompanying tables indicate the relative amount of kidney lipoid stained by Sudan III.

TABLE I—*Guinea Pigs.*

No.	Survival in days	Kidney lipoid
2	Killed at second operation	++
2A	10 (died from overheating)	trace
4	9	++
6	6	++
7	Died from shock, second operation	trace
9	3.5	+++
10	3	++
11	8	++
12	26	+
13	7	++

TABLE II—*Rats.*

No.	Survival in days	Kidney lipoid
3	28	+
11	15	trace
24	9	++
26	12	trace
29	11	++
39	10	+
41	9	trace
51	8	+
52	10	trace
53*	10	++
54	11	+++
56*	13	+++
57	10	++

*Killed.

Eight out of 10 apparently normal guinea pigs serving as controls showed an entire absence of lipoid while one showed +lipoid and the other with chronic diffuse nephritis showed ++lipoid. Lipoid was absent from 3 out of 6 rats used as controls. One showed a trace. The remaining 2 showed + and ++ lipoid, both being rats with sarcoma.

Our observations show that there is a definite increase in the kidney lipoid of guinea pigs and rats after the removal of both adrenals. This increase is not so marked as that present in adrenalectomized cats.

[1] Hartman, F. A., MacArthur, C. G., Gunn, F. D., Hartman, W. E., and MacDonald, J. J., *Am. J. Physiol.*, 1927, lxxxi, 244.

3873

Infectious Dermatitis in Man Produced by a Bacillus Related to Escherichia Formica

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A peculiar skin affection of the wrist in 2 greenhouse workers, father and son, came under our observation, and from the lesions of both patients a bacillus which seems responsible for the affection

was recovered in almost pure culture. At the time of observation the lesions were several months old, having developed slowly in both cases after a diffuse cellulitis of the wrist. The lesions consisted of well circumscribed, round or oval, reddened and indurated, slightly raised patches (2 on the extensor surface of the right wrist in the father and 1 in the same place in the son), of about 2.5 cm. in diameter, presenting a rough surface studded (in the father) with small pustules. Histologically, the lesion was represented by a considerable hypertrophy of the epidermis and a dense cellular infiltration of the corium consisting chiefly of large and small lymphocytic cells, few leucocytes and occasional giant cells of the Langhans type; but in some places there was a predominating leucocytic infiltration with abscess formation.

Smears from the lesions in both cases showed an abundance of Gram-negative bacilli, partly phagocytized and partly free, and a few Gram-positive cocci arranged like staphylococci. In cultures from both cases, on ordinary media, the bacillus grew abundantly and exclusively. From studies of the morphological, cultural, and chemical properties of the microorganisms, it does not seem possible to classify it with any of the known pathogenes. It is pathogenic for rabbits and guinea pigs, especially the latter in which intraperitoneal inoculation of 0.2 cc. of a broth culture caused death with peritonitis, multiple hemorrhages in the lungs, and bacteremia. Injections of killed cultures into rabbits gave rise to the formation of agglutinins and complement fixing antibodies. The bacillus belongs to the genus *Escherichia* (S.A.B.) and but for its failure to grow on sodium formate media and to ferment dulcitol would correspond with *Escherichia formica* (S.A.B.).* However, no group agglutination could be demonstrated with its agglutinating serum on 6 different strains of *Eschericia* (a strain of *Escherichia formica* not being obtainable).

Intradermal injection of 0.2 cc. of a 24-hour broth culture of the bacillus into 4 rabbits and 1 guinea pig caused a circumscribed inflammation (abdomen) which in the guinea pig and 1 rabbit subsided rapidly. In 2 rabbits it persisted for 14 days in the form of a local induration, and in the remaining rabbit the process continued progressively for several weeks in the form of a superficial ulcer, from which the bacilli were recovered in pure culture. Specific agglutinins for the bacillus are present in the father's serum up to the present time, about 1 year after the healing of the lesions.

* Classification Society of American Bacteriologists as given in Bergey's Manual of Determinative Bacteriology, Williams and Wilkins Co., Baltimore, 1925.

The origin of the infection and its relation in the 2 patients could not be established. In several samples of earth and fertilizing material from the greenhouse in which the patients were working, the bacillus was not found. The infection of the son occurred late in the course of the father's infection and the possibility of the son having been infected from the father cannot be excluded.

3874

Elimination of Urine and Dye by Aglomerular and Glomerular Kidneys.

J. GRAHAM EDWARDS. (Introduced by W. J. Atwell.)

From the Department of Anatomy, School of Medicine, University of Buffalo.

Detailed anatomical studies were made preliminary to quantitative analyses of blood (plasma) and urine taken simultaneously from certain fish (teleosts). These studies establish definitely the character of the renal tubule and the blood supply to the kidney. In the aglomerular kidney, the blood supply is solely venous. In the glomerular kidney it is venous and arterial. Although arterial vascularization is apparently the necessary accompaniment of glomerular development, no definite relation obtains between the number of glomeruli developed and the number of tubules connected with them. All stages are found from no glomeruli to few or many. In 4 genera represented in 3 widely differing and unrelated families, the mesonephroi of which are (a) entirely aglomerular, (b) almost aglomerular, (c) predominantly glomerular, the blood and urine were analyzed for the commonly occurring constituents except uric acid and sulphates. The results of these analyses (analyses by Dr. Luigi Condorelli, Department of Clinical Pathology, the Royal University of Naples) show clearly that the urine eliminated by the 3 types of mesonephroi is closely comparable and also comparable to that eliminated by the kidney of higher vertebrates, including man.

The excretion of dye by these kidneys was also determined quantitatively and found to be comparable. As far as could be determined by direct observation of the tubule in the aglomerular kidney of the living fish, which was accomplished with partial success under great difficulty, it appears that the entire tubule is colored by the dye a short time after its injection. Intraperitoneal injections of from 0.6 mg. of the dye, tetrachlorphenolsulphonephthalein, in a 3-gm.

fish to 6 mg. in one weighing 2 kilograms, result in the total elimination of the dye within 15 hours as follows: 1. Aglomerular kidney weighing from 50 to 80 mg.; amount injected 0.6 mg. to 1.2 mg.; 30% elimination by the kidney and 70% in the bile. 2. Almost aglomerular kidney of a 2 to 3 kg. fish; amount injected, 10 mg. intravascularly; amount excreted by kidney within 30 minutes after injection, —20%; bile, none. Further statement to be published elsewhere. 3. Predominantly glomerular kidney; intraperitoneal injection of 6 mg.; weight of fish 2 kg.; amount eliminated by the kidney, —70%; in bile, —30%.

3875

Stone Formation in the Non-Contracting Gall Bladder.

LESTER R. WHITAKER AND DAVID W. PRATT.
(Introduced by W. J. M. Scott.)

From the Laboratory for Experimental Surgery of the University of Rochester School of Medicine and Dentistry.

It has been reported previously that stones could be produced experimentally by interference with the normal mechanism for filling and emptying the gallbladder, resulting in stasis and over-concentration of bile.[1] The observations here presented tend to confirm that finding and add further evidence as to the mode of formation of gall stones.

In one cat while the gallbladder was being filled with iodized oil it was accidentally stripped away from the liver bed nearly down to the cystic duct. The gallbladder containing iodized oil was then replaced in its fossa and the abdomen closed. The next day the viscus had expelled most of the oil and partly refilled with bile, as evidenced by a shadow form with flecks of oil about the sides. The expulsion of the oil was perhaps due to rapid congestion and edema from the injury, which later subsided, allowing partial refilling of the viscus. This shadow form of the gallbladder produced by the radio-opaque oil adherent to its wall remained constant for 11 days, except that it decreased slowly in size to about two-thirds its original volume. A fat meal on the second day produced no change in the shadow. At necropsy the gallbladder was found to be filled with a very hard black cast made up undoubtedly of inspissated bile. The cystic duct, where there had probably been

less stasis, was filled with a black putty-like mass which hardened like the other upon being dried. The vesicle was thin and pale and tense over the stone. Miscroscopic examination showed a good deal of organizing tissue between the gallbladder and the liver. The wall of the viscus and the mucosa, however, were fairly normal, indicating that concentration of bile could have taken place. The whole sequence of events leaves the impression that induced stasis associated with concentration over a period of several days produced a pigment (or mixed pigment) stone.

In another cat the gallbladder was filled with iodized oil in the usual way and the animal fasted for 8 days. For 5 days there was little apparent change in the vesicle. At the ampulla there was a persistent negative shadow indicating incomplete filling with oil, the presumption being that this negative shadow was due to bile. Between the fifth and the eighth day the gallbladder spontaneously emptied its iodized oil into the intestine. Necropsy revealed a somewhat thickened gallbladder containing muco-purulent material and a brownish putty-like ovoid stone packing the ampulla in the exact location of the negative shadow before mentioned. The inference is that stasis of bile in the ampulla of the gallbladder allowed concentration to proceed to stone formation. Why the gallbladder spontaneously emptied after 5 days, during fasting, is unexplained, though it may possibly be accounted for as follows: Denton[2] has apparently shown that impaction of a stone in the ampulla blocks the venous and lymphatic channels, producing hemorrhage and edema in the gallbladder. Possibly this newly formed stone acted in this manner and the resulting thickening of the wall of the viscus with narrowing of the lumen forced the oil out, even past the stone. The process may have been aided by purulent exudation displacing the oil. Microscopic examination revealed pus in the lumen of the vesicle. The mucosa showed normal epithelium with moderate infiltration of poly- and mono-nuclear leucocytes. The muscularis was normal but the subserosa showed mono-nuclear infiltration.

We have never seen this type of stone form in a gallbladder where the epithelium was destroyed, and we do not believe it results primarily from an inflammatory process, unless this inhibits the emptying of the gallbladder by interference with the action of its musculature.[1]

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[1] Whitaker, L. R., *J. Am. Med. Assn.*, 1927, lxxxviii, 1542.

[2] Denton, J., *Arch. Surg.*, 1927, xiv, 1.

3876

Blood Regeneration in Severe Anemia—Influence of Inorganic Ash of Liver, Kidney and Apricots.

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In some of the early anemia work at the University of California Robscheit-Robbins and Whipple observed an unusually favorable reaction to the feeding of dried peaches in short term anemia experiments in dogs. Repitition of this apricot and peach diet in severe anemia in dogs shows clearly that the original observations were correct.[1] The addition of 200 gm. of this cooked fruit to the daily standard diet may cause an average output of 40 to 45 gm. hemoglobin per 2-week period over and above the standard control period. All of this work indicated clearly that the inorganic elements of this fruit should be tested. We have recently reported[2, 3] experiments dealing with various fractions of extracts of beef liver.

It is to be kept clearly in mind that our experiments deal with the simplest form of anemia, due to withdrawal of blood. One must be cautious in comparing simple anemias of this sort with fasting or nutritional anemias. Different animals may react quite differently to various diets—for example, the herbivora may utilize the chlorophyll of green vegetables to build hemoglobin whereas dogs cannot do so.[4] We are greatly interested in a recent preliminary report of Waddell, Elvehjem, Steenbock and Hart[5] which indicates that the ash of beef liver has a favorable effect upon certain nutritional anemia of rats. It is probable that some differences will come out as we learn more about this nutritional anemia as compared with simple anemia due to hemorrhage.

The various samples of inorganic ash were prepared in the following manner: Fresh beef liver, pig kidney, or dried apricots are weighed and placed in a large fire brick container. Free flames from several blow-torches are played on this material until it is reduced to a black carbon mass. At this point a stream of oxygen is directed into the hot material burning it down to a white, gray, or bluish brown, glassy ash. This is then ground in a mortar, passed through a 40 mesh sieve, weighed and fed in doses equivalent to the original fresh material. The material used in these experiments contained 4 to 8% of carbon but it is reasonably certain that all organic com-

pounds are broken down. In liver and kidney ash experiments we used the ash equivalent to 500 to 600 gm. fresh material which varied from 2 to 5 gm. in weight. In the apricot ash experiments the ash equivalent of 200 gm. dried fruits was used—this also varied from 2 to 6 gm. in weight.

Of this fine gray ash 2 to 6 gm. corresponding to a known amount of fresh material were added to the standard basal bread ration[6] of the experimental animal. There was a surprisingly uniform reaction to the various ash samples whether from liver, kidney or apricots. One might suspect the presence of some common substance or group of substances in the material. In fact we are testing various elements and simple compounds which are present in these inorganic mixtures.

We have completed 6 experiments with *liver ash* added to the standard bread ration of these anemic dogs. During the usual 2-week period these dogs averaged 40 to 50 gm. hemoglobin production over and above the control periods. Liver ash corresponding to 500 to 600 gm. fresh beef liver was added daily to the bread ration. These same dogs during a 2 weeks period on 300 to 400 gm. cooked liver, equivalent to 450 to 600 gm. fresh liver, would produce 60 to 100 gm. hemoglobin over control periods.[2] These same dogs on a large excess of ferric citrate (0.2 gm.) or chloride (0.06 gm.) daily during 2 weeks would average about 20 to 25 gm. hemoglobin production over and above the control periods.

We have completed 6 experiments with *kidney ash* using the same amounts as in the liver ash experiments. We may say that the hemoglobin production observed is exactly similar to the liver ash experiments. It may be recalled that cooked pig kidney feeding is about as potent as liver feeding.

We have completed 5 experiments with *apricot ash* which corresponds very closely to those with liver and kidney ash. The average values for the hemoglobin production per 2-week period are 35 to 45 gm. over the control periods. It may be recalled that apricots as fresh cooked moist sauce (200 gm. daily) added to this bread ration produces 40 to 45 gm. hemoglobin over and above the control period. Perhaps one may generalize to the extent of stating that the apricot ash seems to contain about one-half of the potent material in whole apricots favorable to new hemoglobin production.

It would seem that *iron* as the essential factor responsible for the entire reaction could be ruled out on a number of counts. For example, among the fruits tested raspberries were inert, yet they contain as much iron as do apricots which are so active.

It is not easy to understand the mechanism by which inorganic material may so profoundly influence the production of blood hemoglobin. We may recall a few observations which may have some bearing on this puzzle. Fasting dogs usually produce more hemoglobin in anemia than dogs given a liberal amount of carbohydrate, which we believe indicates a careful conservation of intermediates derived from body protein breakdown to be used for construction of new hemoglobin. Further we recall that during a period of rapid gain in weight on a meat diet the dog will not form the expected amount of new hemoglobin. Evidently material suitable for tissue growth has been diverted from new hemoglobin construction. There is probably a certain give and take within the body of essential amino acids and other elements suitable either for tissue growth or repair as well as for new hemoglobin production. It is possible that certain salts and inorganic elements have an influence upon internal protein metabolism and may in some measure determine the direction of the flow of these building stones—now for tissue growth or repair—now for body fluid protein maintenance—now for emergency new hemoglobin and red cell production.

[1] Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Phys.*, 1927, lxxx, 400.

[2] Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Phys.*, 1925, lxxii, 408; *ibid.*, 1927, lxxix, 271.

[3] Whipple, G. H., and Robscheit-Robbins, F. S., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 86.

[4] Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Phys.*, 1925, lxxii, 431.

[5] Waddell, J., Elvehjem, C. A., Steenbock, H., and Hart, E. B., *Science*, 1928, lxvii, 139.

[6] Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Phys.*, 1925, lxxii, 395.

3877

Influence of Acid-Forming and Base-Forming Constituents of Ketogenic Diet Used in Treatment of Idiopathic Epilepsy.

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In a previous communication[1] data were presented to show the relationship between variations in the degree of ketosis and the occurrence of convulsions in certain epileptic children on ketogenic diets. The convulsions were found to occur practically always during the periods of minimum ketosis as measured by the urinary and

blood acetone bodies. However, during the past 12 months, we have repeatedly made the observation recently reported by Lennox,[2] that administration of considerable quantities of sodium bicarbonate to patients under this form of treatment causes temporary recurrence of the convulsions in spite of the diet. This suggested to us the importance of determining the relative efficiency of ketogenic diets having an acid-ash and those containing an excess of base-forming over acid-forming elements.

Observations were made over a total period of 60 days in the case of a 13-year-old patient suffering from severe idiopathic epilepsy. This time was subdivided into periods in which the test diets were given alternately. Except for one period in which a non-ketogenic diet with a very highly acid ash was given, the diets throughout were strongly ketogenic, varying only in their ash content. The most notable fact observed from the study was that the convulsive seizures, which practically ceased to occur during the acid-ash, ketogenic diet periods, promptly recurred in alarming numbers when a diet, containing the same amounts of protein, fat and carbohydrate but with a predominantly basic ash, was given. During the relatively long subperiod, when a non-ketogenic diet with a strongly acid ash was given, the patient had from 2 to 12 grand-mal attacks daily, in spite of the fact that the diet contained in its ash, according to calculation, an excess of acid equivalent to 1110 cc. of 0.1 N solution.

Data derived from a fairly comprehensive study of the factors concerned in the maintenance of the acid-base equilibrium of this patient were found to lend support to the theory that there is a fundamental disturbance in the acid-base metabolism in this form of epilepsy. The ineffectiveness of the ketogenic, alkaline-ash diet in controlling the convulsions is, therefore, probably best explained by assuming that the diet tends to accentuate the abnormal tendency already present. Most of the blood pH values recorded for the periods in which frequent convulsions occurred were found to be definitely above those obtained during the periods characterized by relative freedom from seizures. Three slight convulsions occurred during the first acid-ash period of 11 days, but these were associated in each instance with a marked diminution in the degree of ketosis, following the inadvertent giving of anti-ketogenic material. The time of their occurrence, which was from 1 to 2 hours after meals, and the finding of higher blood pH as well as higher acetone values after meals than before, suggest the possibility of a relationship of these convulsions to the "alkaline tide". This is being investigated

further as a possible explanation for the greater frequency of epileptic convulsions at or near the time of meals.

The fact that the non-ketogenic diet with the excessively acid-ash failed to have any influence on the number of convulsions and failed to sustain the hydrogen-ion concentration of the blood within normal limits, indicates a more or less specific value of the ketogenic diet in this respect so far as its effect on the epileptic subject is concerned. Since the concentrations of acetone bodies in the blood and the urine were found to be elevated during the alkaline-ash period very distinctly above the levels for the acid-ash periods, it is evident that the relationship between the degree of ketosis and the frequency of convulsions previously reported holds only when the ketogenic diet is one with an acid or neutral ash. Finally, it may be tentatively concluded from the foregoing results that the ketogenic diet with an acid-ash is the most efficient so far studied in controlling convulsions in that group of epileptic subjects who respond at all to the dietary form of therapy.

[1] McQuarrie, I., and Keith, H. M., *Am. J. Dis. Child.*, 1927, xxxiv, 1013.
[2] Lennox, W. G., *J. Clin. Inv.*, 1927, iv, 429.

3878

Expulsion of its Contents as a Function of the Gall Bladder.

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Since the introduction of methods that visualize the gall bladder, many opinions have been expressed as to the mechanism of its emptying. These can be divided into 2 groups: (a) that the gall bladder has a passive rôle in this process, and (b) that the gall bladder empties due to the contraction of its own musculature. We wish to present further evidence that expulsion of its contents is an inherent function of the gall bladder and acts independently of purely mechanical factors.

The chief extrinsic agencies suggested as causing the discharge of bile from the gall bladder are: (1) variations in intra-abdominal pressure, (2) intestinal peristalsis, (3) elastic recoil following relaxation of the common-duct sphincter, and (4) the washing out of the gall bladder by hepatic bile.

(1) The fact that the gall bladder may remain full for many days during fasting and that violent struggling associated with tube feeding in the cat produces no discharge of its contents[1] is sufficient evidence that variations in intra-abdominal pressure, within physiological limits, do not cause emptying of the gall bladder.

(2) Vigorous intestinal peristalsis produced by physostygmine as well as the normal movement of a barium meal through the intestine without effect upon the gall bladder containing iodized oil,[1] would seem to rule out intestinal peristalsis as a significant factor.

(3) Though the common duct be completely excluded, a fat meal will induce emptying of the gall bladder through a cannula in the cystic duct (Copher[2]), or the cut end of the common duct (Boyden[3]). Also in one of our cats whose gall bladder had been filled with iodized oil, the hepatic and common ducts were injected from the gall bladder after the ingestion of fat without the discharge of any of the oil into the duodenum. This can scarcely be interpreted in any other manner than as evidence against a reciprocal mechanism and even suggests occasional antagonism of the common-duct sphincter to the expulsive action of the gall bladder.

(4) As Graham has stated, "The real question, however, is not whether contractions occur but whether they are able to empty the gall bladder."[4] He reported that the gall bladder did not empty if the hepatic ducts were occluded and from this he inferred that intrinsic contractions of the gall bladder wall, aside from the factor of elastic recoil, were insufficient to empty that viscus. In fact one of the most recent reports concludes that "muscular contraction is not the only factor involved, and that elasticity and the ebb and flow of fresh bile from the liver play an important part."[2] We accept as critical in the evaluation of these factors the effect of fat feeding on the gall bladder with hepatic bile excluded. Consequently we have repeated this procedure and find that in the cat with all the hepatic ducts tied off (as proven at autopsy) the gall bladder has responded to fat feeding in the characteristic manner by the discharge of over 90% of its contents into the duodenum. These results are similar to those obtained by Higgins and Mann[5] except that the emptying process was carried much further in our experiment, and is obviously impossible of explanation on the basis of mechanical factors such as washing out by hepatic bile or elastic recoil.

We have considered in succession the mechanical processes which have been suggested as causing discharge of bile from the gall bladder and have seen that increased intra-abdominal pressure and intes-

tinal peristalsis under physiological conditions do not produce this effect; that the discharge of material from the gall bladder is even at times initiated against the antagonism of a closed common-duct sphincter and that the usual extensive emptying of the gall bladder proceeds normally in the cat with all of the hepatic ducts tied, far beyond any extent that could be explained on the basis of elastic recoil. The conclusion seems unescapable that the expulsion of its contents in response to fat feeding is a vital function inherent in the gall bladder musculature and independent of extrinsic and mechanical factors.

[1] Whitaker, L. R., *Am. J. Phys.*, 1926, lxxviii, 411.

[2] Copher, G. H., and Illingworth, C. F. W., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 172.

[3] Boyden, E. A., and Birch, C. L., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 827.

[4] Graham, E. A., *Surg. Gyn. and Obst.*, 1927, xliv, 153.

[5] Higgins, G. M., and Mann, F. C., *Am. J. Physiol.*, 1926, lxxviii, 339.

3879

Effect of Thyroxin on Growth Rate and Carbon Dioxide Production of Chick Embryo.

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The data presented here represent the attempt at studies made to analyze the influence of thyroxin upon the pre-natal development of the chick. The technique employed was the same as previously described by the writer[1] for injecting substances into the air sac of the incubating hen's egg. The thyroxin was dissolved in dilutions of sterile distilled water just alkaline (NaOH) to litmus so that the required dose was contained in 0.25 cc. The controls were injected with the same amount of alkaline water.

Experiments were made to determine the appropriate dosage that would be within physiological limits. It is necessary to explain that before the sixth and eighth days of incubation the albumen lies between the air sac and the developing chorio-allantois. Thus an injection placed in the air sac previous to this age would be diluted by its absorption into the albumen. A test dose of 1/300 mg. of thyroxin was given before the start of incubation. This dose proved toxic, so the experiment was repeated giving 1/600 mg. before in-

cubation, and repeating the dose on the thirteenth day. None of the chicks hatched. Then the experiment was again repeated giving only 1/600 mg. on the fourth day with the result that 17 hatched out of 24 incubated eggs. These results indicated that the dose of thyroxin injected into the albumen to be below toxicity must not be over 1/600 mg.

By the sixth or eighth day the albumen is crowded toward the vegetative pole and the respiratory vascular network of the chorio-allantois comes into direct contact with the air sac membrane. From this time until hatching absorption takes place directly into the blood stream. Advantage was taken of this in other experiments and results showed that at this time, the maximum dose compatible with hatchibility is in the neighborhood of 1/40,000 mg. of thyroxin.

The next endeavor was to test the influence of thyroxin upon the growth rate. In order to secure slow absorption and maximum effect 1/600 mg. was injected on the fifth day of incubation. The results are recorded in Table I. Each weight is the average of at least 5 embryos. The mortality was high in the thyroxin series but there is apparently no effect on growth rate. Macroscopic examination of those that died revealed no variation from the normal in size or development. This is significant in view of the results obtained by Willier[2] for thyroid grafts upon the chorio-allantois of the

TABLE I.

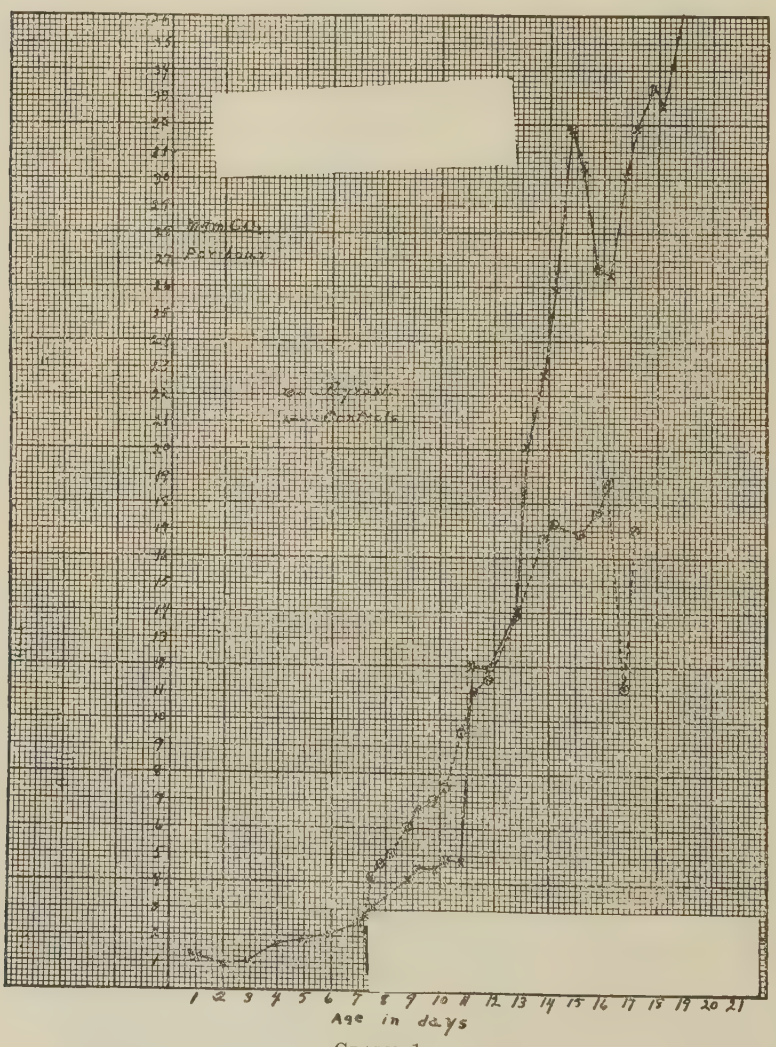
Effect of thyroxin on rate of growth of chick embryo.

Age days	No.	Avg. thyroxin wt.		Avg. control wt.		Mortality	
		Wet	Dry	Wet	Dry	Thyroxin	Controls
5	5	.0397			.00306		
6	5	.16348	.01204	.1205	.00818		1
7	5	.4135	.02602	.3516	.0212	8	3
8	5			.71074	.0456	10	2
9	5	1.0094	.06802	1.03564	.06934	5	2
10						2	1
11	5	2.5573	.18982	2.1935	.16152	2	1
12	5			3.2971	.25502	1	
13	5	5.0178	.4285	5.13708	.4579		1
14				7.22766	.71312	1	
15	5	9.5185	1.48384	9.8966	1.1846		
16							
17	5	16.3158	2.6908	15.5040	2.63804		
18							
19	5	24.3276	4.5119	23.2231	4.4963		
20	5	28.640	5.440	28.120	5.240		
	8	30.2		29.9 forty-eight hours after hatching			

1/600 mg. of thyroxin in 0.25 cc. of distilled water just alkaline to litmus injected into air sac on the 5th day of incubation. Controls injected with 0.25 cc. alkaline distilled water. Average weight of 5 embryos in each series.

chick. He found that the host was always smaller, in some cases one-third smaller, than control.

The CO_2 production was determined by means of an open circuit apparatus using soda lime absorption tubes, similar in principle to the one described by Murray.[3] The average results from 2 typical experiments are shown in Graph 1. The tests in the 2 experiments



GRAPH 1.

Effect of thyroxin upon elimination of CO_2 by the chick embryo. 1/30,000 to 1/40,000 mg. thyroxin in 0.25 cc. alkaline water injected into the air sac on the eighth day of incubation. The controls were injected with the same amount of alkaline water.

varied not over 30 minutes from the same age hour of incubation. The temperature varied between 37°-39° C. Inspection of the control CO_2 curve in Graph 1, which does not vary from the normal, shows that the most easily detected increase above the normal CO_2 production would probably occur between the eighth and twelfth days of incubation. In order to obtain the maximum effect a dose bordering on toxicity (1/30,000 to 1/40,000 mg.) was given just after the control determination of CO_2. As shown, there resulted an increase in CO_2 production extending over a period of about 3 days, then the rate decreased, to remain about the same for 4 days, both embryos dying between the seventeenth and eighteenth days. One of the controls failed to hatch but the CO_2 production remained normal up to the twentieth day. One experiment was carried out using 1/200,000 mg. of thyroxin. The chick hatched but the CO_2 curve did not vary from the normal.

In conclusion, the results showed clearly that when 1/40,000 mg. of thyroxin is introduced into the air sac of the hen's egg at the eighth day of incubation there is a distinct increase in CO_2 production extending over a period of about 3 days. This is followed by a period of marked depression below normal, lasting about 8 days. Doses as small as 1/200,000 mg. produce no demonstrable effect on viability or metabolism. Larger doses (1/30,000 mg. or more) of thyroxin are generally fatal when injected into the air sac at the sixth or eighth day of incubation. A considerably larger amount (1/600 mg.) is tolerated when diluted (possibly modified) by injection or absorption into the albumen at an earlier stage of development, but the period of incubation is not appreciably modified and the weight accretion curve is not altered.

It is proposed to carry out experiments along the same lines with desiccated thyroid gland.

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[1] Hanan, Ernest B., *Am. J. Anat.*, 1927, xxxviii, 423.

[2] Willier, B. H., *Am. J. Anat.*, 1924, xxxiii, 67.

[3] Murray, Henry A., *J. Gen. Physiol.*, 1925, ix, 1.

Pacific Coast Branch.

University of California Hospital, February 15, 1928.

3880

Dormancy or Delayed Germination of Spores of Clostridium Botulinum Subjected to Heat.

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In a previous report[1] it was shown that there is a marked dormancy of spores of *Clostridium botulinum* which have been subjected to heat in broth covered with a thin layer of oil within sealed glass tubes. It has become necessary to discontinue our observations on these tubes and the final available dormancy periods are herewith presented.

These dormancy periods cannot be assumed to be the maximum possible under the conditions of our experiments since positive tubes have continued to appear from time to time until January 1, 1928. No positive tubes were obtained on February 1, but it has not been unusual for intervals of 2 or more months to elapse between the occurrence of positive tubes. The last reading was made on February 1, which is 75 months after the first tubes of the series were heated and 67 months after the heating experiments were discontinued.

In all instances, as was stated in our previous report,[1] the contents of each positive tube were tested by subculture for the presence of living bacteria, and by guinea pig inoculation for the presence of potent toxin, before it was included in our tables.

[1] Dickson, E. C., Burke, Beck and Johnston, *J. Inf. Dis.*, 1925, xxxvi, 472.

TABLE I.

Time of Germination in Months after Spores of Clostridium Botulinum were Heated in Oil-stratified Broth in Sealed Tubes.

Months after heating.	Temperatures at which spores were heated. Degrees Centigrade.					
	100	107	115	118	121	Total
1	1532	521	1039	52	432	3576
2	68	130	312	5	57	572
3	160	124	310	8	49	651
4	126	53	220	—	42	441
5	48	33	121	1	25	228
6	35	11	49	1	18	114
7-9	39	36	98	—	24	197
10-12	9	18	41	—	12	80
13-18	39	16	62	1	27	144
19-21	3	3	2	—	—	8
22-24	15	6	21	1	8	52
25-27	9	4	21	—	5	39
28-30	5	4	8	—	3	20
31-33	2	4	5	—	—	11
34-36	1	2	7	1	1	12
37-39	1	3	9	—	2	15
40-42	4	1	6	—	1	12
43-45	1	—	4	—	1	6
46-48	2	—	8	—	2	12
49-51	—	—	6	—	2	8
52-54	1	—	1	—	—	2
55-57	—	2	2	—	1	5
58-60	—	1	5	—	2	8
61-63	—	—	1	—	—	1
64-66	—	—	2	—	—	2
67	—	—	—	—	—	—
68	—	—	—	—	—	—
69	—	1	1	—	—	2
70	—	—	—	—	—	—
71	1	—	1	—	—	2
72	1	—	—	—	—	1
73						
No. positive	2103	972	2363	70	714	6222
No. heated	3802	2905	14270	490	7954	29421

3881

The Adaptation in Vitro of Diphtheria Bacillus to Specific Antitoxin.

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Observations on the transmutation of toxic and virulent strains of *B. diphtheriae* into atoxic and non-virulent varieties by means of cultivation in a medium containing either normal serum or specific

antitoxin have been reported by Bernhardt,[1] Levinthal[2] and, after the completion of the present work, by Becker.[3] In this paper, which deals with the same problem, the relation between toxin production and virulence of the adapted diphtheria strain was given more attention than has been accorded to it in the past. At the same time the flocculating properties of the atoxic filtrate of an adapted culture when brought in contact with the specific antitoxic serum, were studied in view of the doubt which has recently been expressed in the literature on the specific nature of the flocculation occurring in neutral mixtures of bacterial toxins with their respective antitoxins (Schultz[4]).

Two diphtheria strains, the Park-Williams No. 8 and a recently isolated strain D X, which differed widely under normal standard conditions in toxigenicity and virulence, were employed. The P. W. strain produced a powerful toxin with an m.f.d. of 0.002, while the filtrate of the D X strain obtained under the same conditions yielded a low grade toxin with an m.f.d. of 0.02 (30 cc. of sugar-free veal infusion broth with 0.2% dextrose incolated with one loop of a 24-hour Loeffler slant culture and incubated for 5 days at 35-36° C.). On the contrary, the P. W. strain was fatal for guinea pigs only in amounts as large as ¼ Loeffler slant, while the D X strain was highly virulent, 1/20 slant killing a guinea pig within 31 hours. The antiserum was a nonpreserved diphtheria antitoxic horse serum with a titer of 350 units per cc. Normal horse and tetanus antitoxic horse sera were run along for purposes of control. The inhibitory action of the 3 sterile sera on the growth of the 2 diphtheria strains was determined in preliminary tests. While the antidiphtheric serum showed little or no inhibitory effect on the growth of the P. W. strain, and even in larger amounts inhibited but slightly the D X strain, inhibition of growth of both strains was quite apparent with the 2 other control sera in much smaller doses.

The process of adaptation was carried out as follows: Three sets of broth-serum mixtures were prepared by adding to a constant amount, 5 cc. of broth, 0.3, 0.5 and 1.0 cc. of each of the 3 sera. The two diphtheria strains were cultivated in parallel sets in the mentioned broth-serum mixtures by keeping the cultures for 2 successive passages in the same serum concentration, transfers being made from each tube at the end of 48 hours incubation. Thus after a total of 6 passages the 2 strains grew well in a medium containing 1.0 cc. of antidiphtheric serum in 5 cc. of broth. Daily observations on the activity of growth and on the morphological appearance of the bacilli showed no marked deviation from the

nonadapted cultures. At this stage of the adaptation the 2 specifically adapted diphtheria strains were examined under standard conditions for toxin production, virulence and fermentation reactions, together with the parent cultures which had been carried on simultaneously in broth alone,and with the organisms cultivated in the control serum-broth mixtures. It was found that the 2 diphtheria strains cultivated in the specific antitoxic medium had undergone a profound change in their toxigenic power. This change may be taken to indicate a complete loss of toxin production in view of the fact that the intracutaneous injection into the guinea pigs of 0.1 cc. of a 1:10 dilution, and the subcutaneous injection of amounts up to 2 cc. of the undiluted filtrates, failed to elicit any suggestion of either a local or systemic reaction. On the other hand, the toxigenicity of the 2 strains cultivated in the presence of the control sera had in each instance remained practically unaltered. Subcutaneous inoculations of the organisms into guinea pigs revealed the remarkable fact that all the strains, including the 2 specifically adapted cultures, had not dropped in virulence. Fermentation tests with saccharose, maltose, dextrose and dextrin showed all strains to react true to type.

The atoxic filtrate of the P. W. strain, if given in a single subcutaneous dose of 2 cc., failed to induce the formation of antitoxin in guinea pigs within the period of 3 weeks, as measured by intracutaneous tests. Finally, flocculation reactions were carried out by combining the atoxic filtrates of the 2 adapted strains with diphtheria antitoxic serum according to the method of Ramon. The results of these tests, while not quite constant, demonstrated that the atoxic filtrates flocculated much less than the corresponding potent control toxins. The remarkable fact should be emphasized, however, that some definite flocculation did occur with these atoxic and non-antigenic filtrates.

A continuation of the adaptive process beyond the period indicated led to somewhat irregular results with both the experimental and the control cultures. Further experiments are necessary to determine more accurately whether the atypical properties of the diphtheria bacillus acquired during the exposure to specific antitoxin are permanent or only transitory. The evidence so far seems to point to the latter.

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[1] Bernhardt, G., *Z. f. Hyg. u. Inf. Kr.*, 1916, lxxvii, 179.

[2] Levinthal, W., *Z. f. Hyg. u. Inf. Kr.*, 1926, cvi, 679.

[3] Becker, *Z. f. Immun. Forschg.*, 1927, lii, 402.

[4] Schultz, E. W., *Immun. Forschg. u. Exp. Therap.*, 1928, in press.

3882

Experimental Infection of Dogs with Endamoeba gingivalis and Trichomonas buccalis of Human Mouth.

H. CORWIN HINSHAW.

From the California Stomatological Research Group and the Department of Zoology, University of California.

Neither of the two common protozoan parasites of the human mouth have hitherto been successfully transplanted into the mouths of experimental animals. This has greatly retarded the growth of our knowledge concerning the rôle of these microorganisms in disease.

Our experiments included work with dogs, cats, rabbits, guinea pigs, rats and mice. Attempts were made to introduce cultures of amoebae into the normal gingival sulcus and into artificial extensions of this groove made by means of glass needles and in several cases by means of a chisel shaped knife. In addition to cultures we attempted the introduction of pus directly from a human case of very advanced pyorrhea. Attempts were made to infect guinea pigs which were affected with scurvy. All of the above experiments were negative including those involving normal dogs.

Our first success was with an old dog possessing an advance gingivitis. Several weeks of careful observation demonstrated that there was no spontaneous infection. A culture containing *Endamoeba gingivalis* and *Trichomonas buccalis* recently obtained from a case of active human pyorrhea was inoculated subgingivally on November 6, 1925. Both species continued to multiply in the dog until he was sacrificed 14½ months later. Striking pathological changes were observed which closely simulated human pyorrhea. The greatly accelerated rate of deposition of dental calculus was most marked. This experiment was quite inconclusive as to etiology of the lesions because of inadequate controls.

More recently we have successfully infected 4 other dogs with *Endamoeba gingivalis* after definitely demonstrating the absence of Protozoa before our inoculation. In every case there was more or less inflammation and gingival pocket formation prior to the beginning of the experiment. Only such dogs were susceptible. These and the controls are carefully kept under optimal conditions with properly balanced diets. None of these have borne the infection longer than 4 months. No gross pathological changes have been observed to date and the excellent enviromental factors have caused a systemic improvement in most cases, which is reflected in the dental condition.

3883

Passive Sensitization with Maignon's Fraction of Anaphylactic Blood.*

W. H. MANWARING, J. L. AZEVEDO AND H. C. TORBERT.

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University.

In a recent paper Maignon[1] reports the transmission of acquired hypersensitiveness in dogs by the injection of what we may tentatively call the proteose-peptone-amino-acid fraction of anaphylactic blood. We have attempted to confirm his findings. Maignon drew the blood of horse serum hypersensitive dogs directly into 4 volumes of 95% alcohol, dried and pulverized the resulting coagulum, extracted it with chloroform water, and reprecipitated with 95% alcohol. He obtained from each liter of anaphylactic blood about half a gram of a grayish white product. This product dissolved in physiological salt solution and injected into normal dogs rendered his dogs hypersensitive, the dogs giving apparently classical anaphylactic symptoms on subsequent intravenous injection with horse serum.

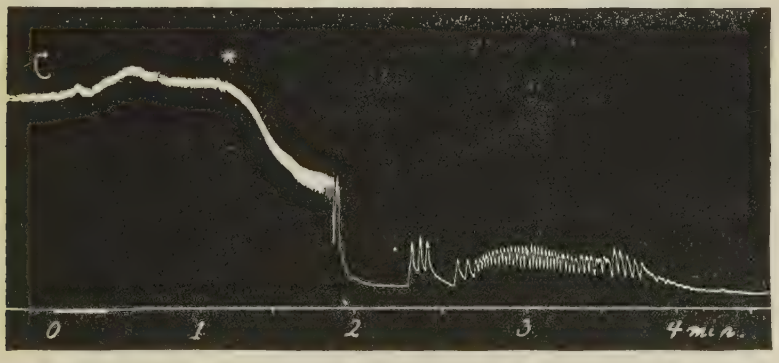

FIG. 1. PASSIVE ANAPHYLAXIS WITH MAIGNON'S PRODUCT.

8 kg. normal dog injected intraperitoneally with 0.75 gm. Maignon's product. Anaphylactic test 24 hours later. Heavy base line, intravenous injection, 8 cc. horse serum. C, changes in carotid blood pressure. *, cessation of respiratory movements.

A second normal dog injected with 0.35 gm. of the same product, gave a milder reaction of the same type; but with respiratory movements resumed at the beginning of the 4th minute and blood pressure restored to normal by the end of 12 minutes.

* Work aided by a grant from Committee on Scientific Research of the American Medical Association.

We have applied Maignon's technic to 9 canine anaphylactic bloods and to emulsions of 6 hypersensitive livers. Eleven of the products thus obtained were wholly inert, giving no suggestion of passive hypersensitiveness even when injected in massive doses. Two of our blood products, however, and 1 liver product gave slight passive hypersensitiveness, and 1 blood product gave the severest anaphylactic phenomenon thus far observed in dogs. A kymograph record with this product is reproduced in Fig. 1.

The symptomatology and autopsy findings with this product, however, were not those of typical anaphylaxis. The outstanding symptoms were a sudden cessation of respiratory movements, accompanied by what was apparently an acute heart block. Death took place in 4 minutes. The precipitous fall in arterial blood pressure, the characteristic feature of typical canine anaphylaxis, was wholly absent in this and in all of our other tests with Maignon's product. On immediate autopsy the splanchnic area was found hyperemic. The hyperemia, however, was of a bright arterial type, not the characteristic cyanotic engorgement of typical anaphylaxis. The liver, for example, was of a bright cherry red color and not appreciably enlarged. On opening the chest, the lungs collapsed normally; no thrombi were found in the heart or in the larger blood vessels; the blood was normally coagulable. Typical anaphylaxis renders canine blood non-coagulable.

The probabilities are, therefore, that with Maignon's product we are not dealing with a true passive anaphylaxis, but with some atypical hypersensitive phenomenon, the nature of which we are at present wholly ignorant.

[1] Maignon, F., *Compt. rend. Soc. de biol.*, 1927, xcvi, 941.

3884

Some Experiments on the Etiology of Diabetes Mellitus.

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From the Department of Bacteriology and Experimental Pathology, Stanford University.

About a year ago, an interesting report was published by Bergey[1] in which the author claimed that he had been able to produce *diabetes mellitus* in rabbits by a single intravenous injection of a Berkefeld filtrate of the urine of diabetic patients. A striking feature in his

observations was the time interval, suggestive of an incubation period, which elapsed between the injection of the urine filtrate and the onset of the glycosuria. Of no little interest also was the observation that filtrates inoculated into serum-broth and incubated for 56 days, or longer, even at room temperature "produced glycosuria in rabbits more promptly and to a more marked degree than freshly filtered urine." The observations suggested to Bergey that the cause of *diabetes mellitus* may be represented by a filtrable, ultravisible organism.

These observations stimulated our interest to the extent that we immediately afterward undertook to repeat these studies. During the course of the work some 30 rabbits were inoculated intravenously with filtrates of diabetic urine in the doses (2 cc.) employed by Bergey. Most of the animals received Chamberland L3 filtrates. Some were injected with L1, others with L2 filtrates, of the same specimen of diabetic urine. Specimens from different patients were employed. The urine samples were collected from the animals by placing them over night in thoroughly cleaned and dry metabolism cages, with no food, but with water before them. The animals were kept on a fairly uniform mixed diet, though this was not strictly standardized. They were for the most part kept under observation for periods of at least 2 months and in some instances as long as 6 months. The urine tests were carried out daily on some animals, others were tested at intervals of several days. All the specimens were tested with both Fehling's and Benedict's solutions (quantitative), both of which had been carefully standardized. Urines giving clearly positive reactions were also submitted to fermentation tests.

The results obtained have been illy defined and entirely unconvincing. Although the urines of some animals showed at times reducing properties, these instances have been comparatively infrequent the reactions have never been pronounced. In several rabbits weak but definite reducing powers were noted within a very few days following the injection, properties which generally disappeared completely within a day or two, either to reappear at isolated times in the future, or never to reappear again during the period of observation. In other animals these isolated, weakly positive reactions were first noted after the second week, in still others not until a later period. Reducing substances to the degree noted not infrequently appeared in the urine of the normal animals before they were inoculated. Fermentation tests in any event were rarely clearly defined so far as gas production was concerned. While the incidence of positive results was somewhat greater in rabbits inoc-

ulated with filtrates passed through the coarser (L1) filters, we are not prepared to say that this fact suggests any relationship to the dimension of the hypothetical agent. Atypical reactions were frequently obtained with Fehling's solution but these are not included in the reactions referred to above. An attempt was made to follow the blood sugar in some of these animals, but the results were too vacillating to be of any value in these studies. We have, therefore, been unable to confirm Bergey's observations. "Cultured" filtrates of diabetic urines were not tried.

[1] Bergey, D. H., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 229.

3885

Bacteriophages From Spontaneous Mouse Tumors.

E. W. SCHULTZ AND K. M. TAYLOR.

From the Department of Bacteriology and Experimental Pathology, Stanford University.

Bacteriophages active for *Eb. dysenteriae* were isolated from 4 out of 8 spontaneous tumors of mice. These were tumors of epithelial origin, which histologically resembled adeno-carcinomata. In size they ranged from 1 to 2 cc. in diameter and, with one exception, were covered with intact skin. They were removed aseptically, finely ground in a mortar under sterile conditions and emulsified in about 10 cc. of physiological saline. The emulsions were immediately filtered through sterile filter paper and then through a candle. These filtrates were tested in the customary manner for lytic action against strains of *Eb. dysenteriae. Eb. typhi, Es. coli* and of *S. aureus.* Several serial passages sufficed to elicit the presence of a bacteriophage active for *Eb. dysenteriae.* By the tenth passage a few drops of the filtrate was sufficient to lead to complete lysis of an actively growing broth culture of the organism. Typical plaques were produced on solid media. No detectable lysis was produced in the presence of the other bacterial species named. One strain of *S. aureus* was, however, strongly agglutinated by the active filtrates. The agglutinating principle was, moreover, transmitted in series by means of filtrates of the successively agglutinated cultures. That this was not a natural property of the organism was indicated by the appearance of the control cultures. While this "transmissible agglutinin" presumably represented a weak bacteriophage for the

organism, it is curious that no appreciable lysis could be detected in the broth cultures during the course of these serial passages. At the end of the 25th serial passage the picture was essentially the same as during the earlier passages.

With the exception of one tumor presenting surface ulcerations, from which a mixed bacterial flora was cultivated, cultures made from the freshly harvested tumor pulp failed to reveal the presence of bacteria in the tumor tissue. It would for this reason be difficult to explain the presence of the bacteriophage in these tumors were it not for the fact that a similar bacteriophage may also be isolated from the intestinal contents of such mice. It is entirely conceivable that it may find its way from there to various tissues of the body.

3886

Reciprocal Innervation of Antagonistic Eye Muscles.*

O. L. HUDDLESTON AND H. E. DE FEO. (Introduced by T. C. Burnett.)

From the Rudolph Spreckles Physiological Laboratory of the University of California, and the University of Colorado Medical School†

Reciprocal innervation of antagonistic eye muscles has been more or less generally accepted since the time of the experiments of Sherrington.[1] Recently, however, some controversy has arisen, due chiefly to the experiments of Tilney and Pike[2] and of Lorente de Nó.[3] These investigators have concluded that the law of reciprocal innervation is not valid in the case of antagonistic eye muscles. It therefore seemed to us desirable to further investigate this problem. The animals we used in our experiments were dogfish *(Mustelus californicus* and *Triakis semifasciatis)*. In order to demonstrate the phenomenon of reciprocal innervation we have employed a slightly different method from any used heretofore. Instead of stimulating the cerebral hemisphere or rotating an animal around one of its principal body axes to evoke eye movements, we employed the method used by Maxwell.[4] Briefly, this method consists in exposing a semicircular canal and subsequently applying a mechanical stimulus to its ampulla.

* The expenses of this research were defrayed by a grant from the Board of Research of the University of California.

† The experiments were conducted at the Scripps Institution of Oceanography of the University of California, La Jolla.

Labyrinthine excitation invariably results in conjugate movement of the eyes, the direction of which depends upon the particular structure which is stimulated. When a stimulus is applied to the ampulla of the right horizontal canal, the eyes turn to the left in the horizontal plane. Stimulation of the left horizontal ampulla causes both eyes to turn to the right. The most important muscles concerned in the production of ocular rotation in the horizontal plane are the *rectus internus* and *rectus externus.* In order to test the reciprocal action, these muscles were detached from the bulb of the right eye and were connected by means of threads to recording levers (modification of the Bartels[5] method used by Maxwell and Huddleston[6]). Graphic records were made of the responses of these muscles to alternate stimulation of the right and left horizontal ampullae.

When a stimulus is applied to the ampulla of the right horizontal canal, the *rectus internus* of the right eye strongly contracts and the *rectus externus* relaxes. Stimulation of the left horizontal ampulla causes the *rectus externus* to contract and the *rectus internus* to relax. The extent of relaxation of the antagonistic muscle, however, is never found to be proportional to the contraction of the protagonist, and indeed may be entirely absent in many cases. When an antagonist fails to relax under these conditions, does it mean that reciprocal innervation of these muscles is absent, or is it that the conditions are not optimal for the appearance of the phenomenon?

When an extrinsic eye muscle is caused to contract by labyrinthine stimulation it slowly relaxes, requiring 4 or 5 seconds at times for it to return to its original position. This means of course, that the muscle, during the period of relaxation, is in a state of gradually diminishing tonic contraction. Thus if the *rectus externus* is caused to contract by stimulating the left horizontal ampulla, it will slowly relax when the stimulus is removed from the ampulla. If now, a stimulus is applied to the right horizontal ampulla during the period of relaxation, the muscle relaxes rapidly, and simultaneously with the contraction of the *rectus internus.* When either of these 2 muscles is caused to enter into a state of tonic contraction by stimulating the appropriate ampulla, it invariably relaxes almost instantaneously when the opposite horizontal ampulla is stimulated; the onset of relaxation of the antagonist coincides almost exactly with the beginning of contraction of the protagonist. We, therefore, interpret these results as proof of reciprocal inneraction of the *rectus externus* and *rectus internus.* At a future date we are planning to test the

reciprocal relation of the 2 remaining pairs of antagonistic eye muscles.

[1] Sherrington, C. S., *Proc. Roy. Soc.*, 1893, liii, 407.

[2] Tilney, F., and Pike, F. H., *Arch. Neurol. and Psychiat.*, 1925, xiii, 289.

[3] Lorente de Nó, R., *Trav. Lab. recherches biol. univ. Madrid*, 1925, xxiii, 259.

[4] Maxwell, S. S., "Labyrinth and Equilibrium," Monographs on experimental biology, J. B. Lippincott Company, Philadelphia and London. 1923.

[5] Bartels, M., *Arch. Ophthal.*, 1911, lxxciii, 129.

[6] Maxwell, S. S., and Huddleston, O. L., *J. Gen. Physiol.*, 1926, viii, 441.

3887

Effect of Insulin on Protein Metabolism.

VEON CARTER KEICH AND JAMES MURRAY LUCK.

From the Department of Chemistry, Stanford University.

The effect of insulin upon the protein metabolism of the rat was investigated by analyzing the entire carcass for urea and amino acid nitrogen within 1 to 4 hours after the commencement of the experiment.

Each estimation consisted of the sum of the substance in question within the animal and that amount which had been excreted within the experimental period. The values obtained were compared with those from control animals which received injections of 1% sodium chloride. Marked increases in the rate of urea formation were observed. The amino nitrogen content of the whole animal decreased. The average decrease observed was approximately equal to the average increase in urea nitrogen. These results were found to be independent of the nature of the diet—high or low protein, upon which the animals had been maintained for the preceding 3 days.

It is possible that the increased protein catabolism, here observed, is secondary to the accompanying hypoglycemia, and is due to a compensatory increase in the rate of glucogenesis from amino acids.

3888

Occurrence of Deciduomata in Rats Low in Vitamins A and E.

K. SCOTT BISHOP AND AGNES FAY MORGAN. (Introduced by E. T. Engle.)

From the G. W. Hooper Foundation and the Department of Household Science, University of California.*

In one rat of a series maintained on A low diets, multiple deciduomata occurred spontaneously. There were 12 macroscopic tumors in the right horn and 11 in the left, while the right ovary sectioned shows 5 large corpora and the left ovary 7. Sections show microscopic tumors between the larger ones. This rat was never mated. At 5 months of age it was transferred from a diet low in A to one free from A (extracted gluten and casein—cornstarch—lard—salts —yeast). This diet is also low in E. Cornification of the vaginal cell content became complete at once (Evans and Bishop,[1] Wolbach and Howe[2]), and persisted for 15 days when 2 drops of cod liver oil were given daily, producing a normal vaginal cell content, although no oestrus occurred after the cure. Fourteen days later vaginal blood was noted and autopsy performed.

In another series of E free rats, we have noted persistence of the decidual reaction in those showing resorption of embryos before the tenth day of pregnancy, either when A also is low, or when several matings, with histories of resorptions, have followed each other without rest periods. However, none have shown the generalized and spontaneous reaction found in the first case. It seems possible that the causative factor there was the curative effect of the cod liver oil acting on foci of keratinization in the uterine mucosa, together with the chance occurrence of a single ovulation cycle.

* Aided by grant from John C. and Edward Coleman Memorial Fund.

[1] Evans, H. M., and Bishop, K. S., *J. Metabol. Research*, 1922, i, 343.

[2] Wolbach, S. B., and Howe, P. R., *J. Exp. Med.*, 1925, xlii, 753.

Illinois Branch.

Northwestern University, February 28, 1928.

3889

Development of Argyrophile and Collagenous Fibers in Tissue Cultures.*

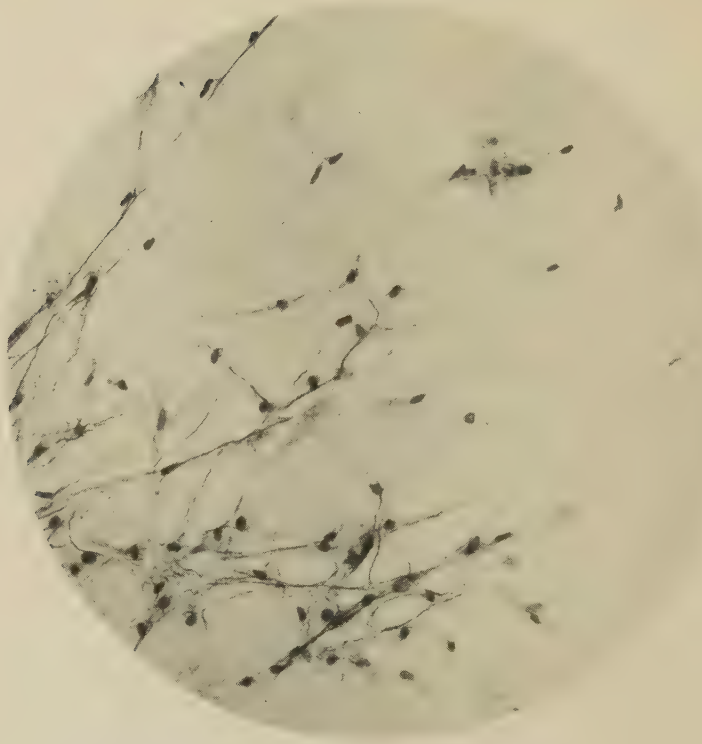
ALEXANDER MAXIMOW.

From the Department of Anatomy, University of Chicago.

In cultures of adult connective tissue of the rabbit which grow undisturbed on slides or in flasks in the usual plasma and embryonic extract medium for a period of 2 to 3 weeks, development of a fibrous intercellular substance can be observed in the living condition as well as after fixation and staining. In the first stages of this process, extremely delicate loose networks of thin, wavy, branching and anastomosing fibrillae appear around the isolated star shaped fibroblasts which advance into the nutritive medium. The fibrillae are argyrophile, *i. e.*, they are electively impregnated with silver. Although in many places they follow the outlines of the fibroblasts and their processes, and even adhere closely to their protoplasm, the fibrillae are also seen from the very beginning to extend far into the surrounding medium. As the outlines of the fibroblasts are always very distinct, the existence of an exoplasm can be excluded with certainty. The fibrillae sometimes seem to follow the course of the threads of the fibrin network. A direct transformation of the latter into argyrophile fibrillae, however, cannot be proved. (Fig. 1.)

In later stages, which can be observed in the inner areas of the zone of newly formed tissue, the argyrophile fibers become thicker and more numerous, and form dense networks. They continue to adhere to the surface of the fibroblasts, so that the cell bodies often seem to be surrounded by black, basketlike networks. The spaces between the cells are also penetrated by an increasing number of

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.



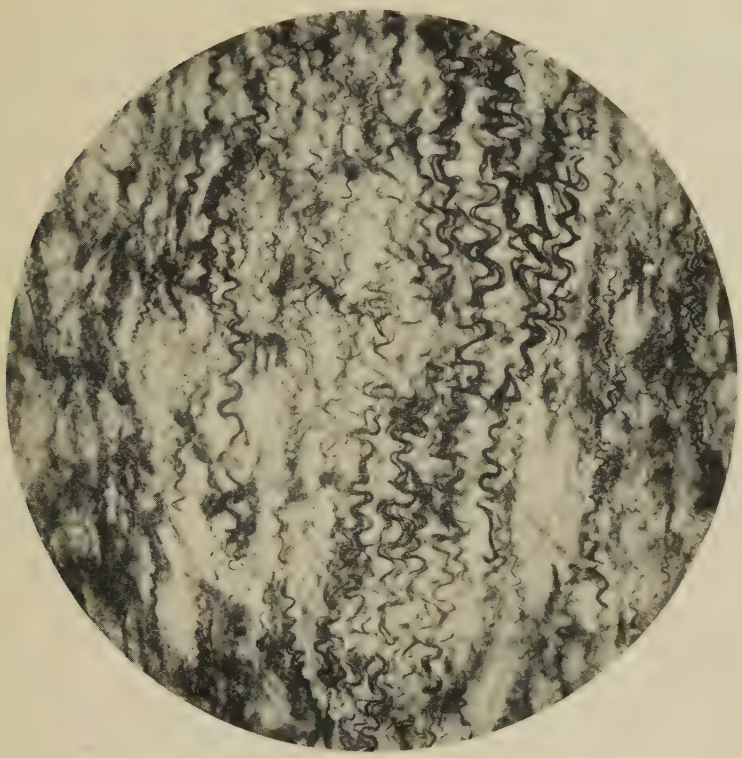
MICROPHOTOGRAPH 1.

Culture of connective tissue of 21 day (3 transplantations). Formation of argyrophile fibrillae around and in connection with fibroblasts. 200 x.

branching argyrophile fibers connected with the pericellular networks. If epithelial strands are present in the culture, as, for instance, in the cultures of the thymus, dense argyrophile basement membranes appear on the surface of the epithelium.

The branching and interlacing fibrillae gradually become arranged in parallel, longitudinally striated strands or bundles, which stretch in various directions and are connected everywhere by looser networks of argyrophile fibrillae. The heavier bundles are quite independent from the cell bodies; the latter may be only adjacent to their surface. In cases where the fibrin layer, in which the culture is growing becomes detached from the bottom of the flask and contracts, the strands of argyrophile fibers acquire a typical, regular, wavy appearance.

In the later stages the quantity of the fibrous substance continues to increase and the bundles become heavier. Simultaneously the fibers in the thicker bundles lose their argyrophilia and become



MICROPHOTOGRAPH 2.

Culture of connective tissue of 45 days (7 transplantations). Formation of wavy collagenous bundles from argyrophile fibrillae. 320 x.

acidophilic. After silver impregnation they are brown instead of black; with the van Gieson or Mallory stain they are intensely red or blue respectively (Fig. 2.) Everywhere in the newly formed tissue gradual transitions from black to brown (red or blue) wavy fibrillar bundles can be observed. Thus, the fibrous intercellular substance acquires all the morphological properties of true collagen. In the latest stages observed (30-50 days) large areas in the cultures were occupied by sheets of heavy, regularly arranged, wavy collagenous bundles with cells scattered between them. Such a tissue looked exactly like a small tendon or a fibrous membrane, as found in the body.

That the fibrous substance was true collagen was shown by the digestion test. In alcohol fixed material an alkaline pancreatin solution dissolved the cells and the fibrin, but left the fibrous masses unchanged.

It may be added that the same formation of fibers was also ob-

served in cultures of connective tissue, which were obtained from the buffy coat of centrifuged blood of the guinea pig.

Thus, abundant formation of true collagen can be obtained in cultures of adult mammalian connective tissue. The process follows the same paths as in the body. At first argyrophile fibrillar networks appear which in every respect are identical with the so-called reticulin or lattice fibers ("Gitterfasern"). Later, with continued increase in quantity, the fibers become arranged in parallel, wavy bundles, lose the argyrophilia and begin to stain in the fashion of mature collagen. Nothing could be observed which would substantiate the idea of the transformation of the cellular protoplasm or exoplasm into reticulin or collagen. The first fibrillae appear in the medium surrounding the cells, as the result of precipitation or, perhaps, of transformation of some colloidal sol into a gel under the influence of an unknown factor, probably of chemical nature, which originates in the cell body of the fibroblasts and diffuses into the surrounding medium. Therefore the fibrillae first arise in the immediate neighborhood, sometimes directly on the surface of the protoplasm. They, however, extend at once far into the medium, away from the cells. It is probable, that, as Heringa and Lohr[1] suggested, the particles of the colloids in question are rod shaped. This causes the gel to assume a fibrillar structure. The fibrin threads of the plasma clot seem to serve as pathways for the precipitating material. Whether the fibrin itself is transformed into reticulin and later into collagen (Baitsell[2]) is doubtful.

[1] Heringa, G., and Lohr, H., *Kon. Akad. v. Wetensch. te Amsterdam,* 1926, xxvi, 1081.

[2] Baitsell, G., *J. Exp. Med.,* 1915, xxi, 455.

3890

Symptoms Resembling Epilepsy Following Experimental Lesions in Brain of the Dog.

LAWRENCE O. MORGAN AND CLARENCE A. JOHNSON.
(Introduced by V. E. Emmel.)

From the Department of Anatomy and Laboratory of Physiological Chemistry, University of Illinois College of Medicine.

This study is based on 16 dogs in which a lesion was successfully placed in the mammillo-infundibular region of the diencephalon.

Symptoms were recorded, blood chemistry studied and the brains prepared for microscopic study.

Apparently normal in other respects, these animals had periodic fits which in all essential respects were identical with the characteristic epileptic fit in human. The animal would suddenly become rigid and plunge forward or fall on its side. The rigidity was followed by convulsive spasms of all voluntary muscles. The fit was further characterized by unconsciousness, dilation of pupils, frothing at the mouth, marked vasoconstriction, erection of the penis, increased heart rate, rise in temperature, frequently by micturition and occasionally defecation. The fit itself usually lasted from 1½ to 3 minutes and left the animal confused and disorientated for several minutes. In some cases the fits would begin rather lightly a few hours after the operation and would occur at intervals of 40 to 60 minutes. These fits would then become more frequent and more severe until the animal passed into a continuous state of coma with convulsions occurring every few seconds and death would soon follow. At this later stage the heart rate increased to from 200 to 250, the temperature rose to 105°-110° and in 3 cases dilation of the intestines and stomach and contraction of the bladder were noted. A second group of animals had periodic fits for 1 to 3 days following the operation and then recovered.

The cardiovascular disturbances, inhibition of gastro-intestinal musculature, dilation of pupils and salivation are suggestive of hypersecretion in the suprarenal glands. The thyroid and parathyroid may be concerned with the symptoms involving the voluntary musculature. The lesion in these cases involved the substantia grisea of the third ventricle, the mammillo-infundibular nucleus, the nucleus tuberis, and frequently but to a less degree, the medial cells of the basal optic ganglion. The first 3 of these nuclei occupy a considerable area lateral to the third ventricle, from the optic chiasma to the mammillary bodies and extending dorsad to the thalamus. The substantia grisea lies mediad, the mammillo-infundibular nucleus laterad and the nucleus tuberis ventrad, in this region. The cells of these 3 groups intermingle freely ventral and medial to the fornix. The basal optic ganglion extends above the optic tract and the more medially placed cells lie in close proximity to the area occupied by the other 3 nuclei. These nuclei probably receive fibers from both the cerebral cortex and corpus striatum.

The lesions were made by the injection of 2-3 drops of a 0.2%-0.5% solution of mercuric chloride. It is believed by the authors that the symptoms are caused by an irritative stimulation of the

cells in the area involved rather than inhibition of the normal activity of these cells.

A study of blood chemistry is being made but more data are required before the findings can be reported.

3891

Localized Cell Destruction and Degenerative Processes in the Brain in Idiopathic Epilepsy.

LAWRENCE O. MORGAN. (Introduced by V. E. Emmel.)

From the Department of Anatomy, University of Illinois College of Medicine.

This study is based on 4 brains from patients which were diagnosed as typical cases of idiopathic epilepsy. Celloidin sections were made through the region of the third ventricle and stained with iron-hematoxylin and iron-hematoxylin and neutral red. There is a marked localized distention of the third ventricle from the optic chiasma to the mammillary bodies and extending dorsad to the intermediate commissure of the thalamus. This distortion is due to a shrinkage of tissue in the lateral walls of the ventricle. There is a marked hyperemia confined to the region of the third ventricle.

The following cell groups are affected:

(1) The substantia grisea of the third ventricle is composed normally of small rather closely packed nerve cells in the lateral wall of the ventricle from the optic chiasma to the mammillary bodies and extending upward to the thalamus. These cells mingle laterally with those of the mammillo-infundibular nucleus. In all of the 4 brains studied there is a marked bilateral reduction in the number of cells in this group. Many of the remaining cells show various stages of chromatolysis. There is a marked proliferation of glia cells in some areas. A shrinkage in this cell mass is largely responsible for the distention of the third ventricle.

(2) The mammillo-infundibular nucleus is normally composed of large scattered cells more laterally placed than the substantia grisea and extending from the infundibular region to the caudal level of the mammillary body. The cells are most concentrated around the fornix and lateral to the mammillary body. The cells of this nucleus are considerably reduced in number in the epileptic brains, both sides being affected. A great many of the remaining cells show chromatolysis. In the areas most affected there is

marked proliferation of glia cells which are sometimes seen to invade the dying nerve cells.

(3) The nucleus tuberis is composed of rather large elongated cells arranged into 3 or more groups. These groups of cells lie close to the basalar surface, ventral to the fornix, and extend from the infundibulum to the mammillary body. This nucleus shows considerable cell destruction in all of the epileptic brains. A great many of the remaining cells are chromatolytic. The medial cell groups are usually affected more than the lateral.

(4) The basal optic ganglion is composed of a group of large cells flattened over the lateral, dorsal and medial surfaces of the optic tract near the chiasma. In the epileptic brains studied this nucleus shows a varying amount of chromatolysis but very little if any cell destruction.

The cell groups most markedly affected in the 4 epileptic brains studied are the substantia grisea of the third ventricle, the mammillo-infundibular nucleus, and the nucleus tuberis. It is believed by the author that these are centers for the control of the thyroid, parathyroid and suprarenal glands. The basal optic ganglion which seems to be concerned to a less degree with epilepsy has been shown by Greving to have fiber connections with the hypophysis.

These findings suggest the possibility that epilepsy may be the result of a functional disturbance of the glands of internal secretion, probably a hypersecretion caused by irritation of the nervous centers in the diencephalon.

3892

The Effect of Yeast Upon Metabolism.

EUGENE U. STILL AND ELIZABETH M. KOCH.

From the Physiological Chemistry Laboratories, University of Chicago.

Although several workers have investigated the effect on the metabolism of human subjects of adding yeast to a diet in such quantities as are commonly recommended for therapeutic purposes, the results reported have shown considerable variation. Further studies, with and without yeast, seemed important both for confirmation and additional information.

Plan of Experiments: Table I shows the general plan of the 3 experiments. We studied our several subjects over longer periods

of time than most workers. Breakfasts and dinners were eaten in the home of one of the subjects. Luncheons were eaten in the laboratory. The food was weighed and liquids measured.

TABLE I.

Exp. No.				
1 Low protein Low purine	3-3 day periods No yeast 3 day analyses	2-4 day periods Plus yeast* 4 day analyses	2-4 day periods No yeast 4 day analyses	
2 Same	3 weeks on unweighed diet	11 days on weighed diet—no yeast 3 day analyses on last 9 days	24 days on same diet plus yeast No analyses	11 days on same diet plus yeast 3 day analyses on last 9 days
3 High protein High purine	3 weeks on unweighed diet plus yeast No analyses	7 days on same diet but weighed No analyses	3-2 day periods 2 day analyses	3-2 day periods No yeast 2 day analyses

*During the yeast periods, one cake of yeast was ingested at each meal.

The urines were analyzed for volume, specific gravity, total nitrogen, ammonia plus urea nitrogen, uric acid, creatinine, total phosphorus, glucose, total phenols, acidity and formol titration. Blood was analyzed for N.P.N., urea nitrogen, uric acid, creatinine, total phosphorus and glucose. The feces were analyzed for total weight, moisture, total nitrogen and total phosphorus.

Results: During the yeast ingestion there was an increased excretion of nitrogen and phosphorus (sum of urinary and fecal). The larger share of this excess excretion above the control periods was found in the feces, indicating a poor utilization of yeast nitrogen and phosphorus.

The total urinary phenols were less during and following the yeast periods in 5 of our 6 subjects. This seems significant, especially because the added yeast contained considerable tyrosine which is a possible source of phenols. This decrease seems to suggest a decreased intestinal putrifaction brought about by a change in the intestinal flora.

The addition of yeast to a low protein, low purine diet after the subjects have arrived at a low uric acid elimination caused no increase in uric acid excretion. If the yeast was added while the uric acid elimination was still high, or, while the subjects were on a high protein, meat diet, an increased uric acid elimination promptly followed. When yeast was discontinued after high uric acid excretion, the uric acid excretion fell off promptly. There was no increase in blood uric acid even after several weeks of yeast ingestion.

While yeast generally produced a greater regularity and ease of evacuations, the moisture of the feces did not seem to be noticeably or consistently increased. The yeast feces were more bulky and easier to mix and sample. This change in the character of the feces was probably the result of yeast fermentation and greater porosity.

There were no consistent changes which could be correlated with the ingestion of yeast for the blood and urine glucose, creatinine, acidity, urea, etc.

In our study, the changes following the ingestion of one cake of yeast per meal permit the following conclusions: 1. The yeast had little effect on nitrogen metabolism. Most of the added yeast nitrogen was excreted in the feces. 2. The yeast had little effect on phosphorus metabolism. Most of the added yeast phosphorus was excreted in the feces. 3. The yeast protein does not seem to be well utilized. 4. There is no retention of uric acid following yeast ingestion as is evidenced by no change in the blood uric acid. The ingestion of yeast does not cause an increased excretion of uric acid unless the level of uric acid excretion is already high, then the ingestion of yeast causes an increased excretion of uric acid which promptly falls off when the yeast is discontinued. 5. The ingestion of yeast caused a change of intestinal flora as evidenced by the reduction of urinary phenols. 6. The ingestion of yeast caused no consistent changes in the moisture content of the feces; however, the greater bulk and porosity due to fermentation caused evacuations to be easier.

3893

Studies on Hemoglobin Formation in the Rat.

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The purpose of these experiments was to study the relationship between the protein and vitamin composition of the diet and the hemoglobin forming process in the rat. In a study of this type, hemoglobin estimations and red corpuscle counts are of diminished significance unless the blood volume factor is controlled. For control of this factor we have developed a micro-modification of the Kieth-Rowntree plasma-dye method which makes possible repeated blood volume determinations in the rat. The method tested upon measured samples of blood *in vitro* shows an error not exceeding

4%. Applied to the rat the method yields consistent and reproducible results. With proper manipulation the mortality does not exceed 5%.

In this work 2 experimental methods have been applied. The first is of the type commonly used for anemia studies in rats with the additional precaution of determining blood volumes. Young, growing rats were fed upon various synthetic diets and the changes in hemoglobin, red corpuscle count, and blood volume recorded over periods of 8 to 12 weeks. The changes in the number of grams of circulating hemoglobin per 100 gm. body weight are taken as an index of the rat's ability to form hemoglobin upon the diet under consideration.

In later work we resorted to a severe anemia type of experiment similar to that used by G. H. Whipple[1] and his co-workers upon the dog. The hemoglobin level is reduced to approximately half its normal value and maintained at this level by repeated bleedings. The rat is bled by cardiac puncture under ether anesthesia. If, over any period of time, the blood volume and hemoglobin concentration are the same at the beginning and end of the period, the amount of hemoglobin removed by bleeding during this time represents the amount of hemoglobin produced by the rat over and above the maintenance factor. Thus, this second method enables us to measure the rate of hemoglobin formation in the rat upon various diets and under conditions of maximum stress.

Using the first type of experimental method described above, the possible relation of tryptophane to hemoglobin formation was studied. As a control for tryptophane feeding a synthetic diet was used containing 10% of wheat gluten. The mineral content of all the diets used in this work was complete and adequate since we wished to study only organic factors. A tryptophane analysis on the gluten showed that the rats upon the control diet received less than 5 mg. of tryptophane daily. The rats upon this diet did not become anemic and an increase of the tryptophane consumption to 65 mg. per rat daily failed to increase their ability to form hemoglobin.

In another series of rats, casein was compared to gluten and was found to be no better utilized by the rat for hemoglobin formation than was gluten. The effect of feeding red blood corpuscles was also studied using the 10% gluten diet as a control. The results were negative.

Using the severe anemia type of experiment described above, the rate of blood regeneration upon the 10% gluten diet was determined. It was found that under these conditions the rats regenerated the total hemoglobin content of their blood every 10 to 13 days,

and maintained this high rate of blood formation for weeks. Upon a casein diet the rate of blood regeneration was found to be no higher than upon the gluten diet. Feeding red corpuscles to the extent of 15% of the diet failed to increase the rate of blood formation over that of a control period.

The relations of vitamin A, B, and E to hemoglobin regeneration were studied, using the severe anemia type of experiment. It was found that rats in a marked state of avitaminosis due to the long continued absence of vitamins A, B, or E in the diets could regenerate their blood at the normal rate. Furthermore, the addition of vitamins A, B and E to diets deficient in these substances failed to produce any effect upon the rate of blood regeneration.

Conclusions: 1. Wheat gluten is an adequate dietary protein for promoting hemoglobin synthesis in the rat. Casein is not superior to wheat gluten for this purpose. 2. Hemoglobin and tryptophane in the diet are no better utilized than gluten for hemoglobin production in the rat. 3. The blood forming process in the rat is not dependent upon the presence of vitamins A, B, or E in the diet.

[1] Whipple, G. H., *Am. J. Physiol.*, 1925, lxxii, 395.

3894

Rate of Liberation of Tryptophane from Proteins by Enzymes.

IDA KRAUS-RAGINS. (Introduced by F. C. Koch.)

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Casein, edestin, Witte peptone and squash seed globulin were subjected to trypsin hydrolysis. At different intervals of time a portion of the respective hydrolysates was taken and tryptophane determined by the indirect Vanillin-HCl reaction.[1] At the end of one hour three-fourths of the total available tryptophane in casein was liberated, a little less than one-half was liberated from edestin and two-fifths from squash seed globulin. Witte peptone had one-third of the total tryptophane available before incubation with trypsin and at the end of the first hour two-thirds was available. Equilibrium was established in the case of Witte peptone in 24 hours, casein in 72-96 hours, edestin and squash seed globulin in 120 hours. The latter 3 proteins were subjected to the action of pepsin, trypsin and erepsin in the order given and aliquot portions were taken and analyzed for amino nitrogen and for tryptophane.

At the end of the pepsin period of 96 hours there was an average of 17% amino nitrogen but no tryptophane liberated. During the trypsin period the liberation of tryptophane and the establishment of equilibrium was the same as in the experiment with trypsin alone without any pepsin action. Erepsin action for 48 hours showed an additional liberation of 15-18% of amino nitrogen but no change in the tryptophane concentration. Thus, trypsin or a trypsin type of enzymes alone are involved in the liberation of tryptophane from the proteins studied.

The effects of sodium and chloride ions on the precipitation of tryptophane by mercuric sulfate were studied. A 0.3% concentration of chloride ion interferes with and a 0.77% entirely prevents the precipitation of tryptophane under the conditions as given in the indirect Vanillin-HCl reaction. Sodium in concentrations up to 2% has no effect.

[1] Kraus, Ida, *J. Biol Chem.*, 1925, lxiii, 157.

3895

Demonstration of Rapid Pepsin-Hydrochloric Proteolysis in Vitro.

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Hydrolysis of the more complex protein molecule into simpler forms carried on with the aid of proteolytic enzymes ordinarily requires considerable time even at body temperature. Some time ago it was observed that the addition of a very small amount of solid pepsin to fibrin jelly causes an almost immediate solution of the fibrin at room temperature. The jelly was produced by treating 20 gm. of washed fibrin with 250 cc. of .04% hydrochloric acid. If the jelly is stirred or shaken after the addition of the pepsin, 5 minutes usually suffices to put the fibrin into solution. An observation on this point is recorded in literature but no definite data is given as to condition of the experiment, the speed of the solution or the nature of the end products.

Recently the nature of the soluble protein products has been studied. Dr. Hektoen determined by means of an anti-fibrinogen serum that the soluble protein, resulting from this treatment, was immunologically different from fibrinogen. This result definitely

eliminated the idea that this reaction might be a reversal of the one which causes conversion of fibrinogen into fibrin. A further study on the soluble products resulting from this treatment revealed the fact that they are largely proteose in character and that the larger percentage of the compounds falls between the limits of 50 to 100 saturation with ammonium sulphate. We have then, here, a definite demonstration of extremely rapid proteolysis at room temperature.

3896

Some Effects of Histamine on the Acid-Base Balance.

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The "alkaline tide" in the urine during the digestive period is commonly regarded as a compensatory process, eliminating the excess of base left in the blood after the formation of HCl in the stomach. Some of the observed variations in urine reaction, however, may be indirectly dependent on the absorption of food. So far as we are aware the alkaline tide has not been shown to accompany gastric secretion in the fasting subject. Ackman[1] studied the urine reaction in man, giving a test meal followed immediately by the administration of histamine. The food factor was therefore not eliminated. It seems that the use of histamine alone might give more valuable information as to the cause of the tide.

Female dogs, weighing from 9.0 to 12.5 kg., and having fasted for 16 to 20 hours, were used in our experiments. Measurements of pH were made on urine by the potentiometer, and on blood plasma by the method of Hastings and Sendroy.[2] In the studies on urine, each animal was as a rule used for a single experiment of 4 to 6 hours, in order to avoid the effects of a possible cystitis from the use of the catheter. Histamine dichloride was injected subcutaneously, the usual dose corresponding to 0.7 mg. of histamine base.

Such a dose of histamine invariably produces a rise in urinary pH, lasting for about 2 hours, after which the reaction returns to near the original level. The duration of this effect suggests that it is related to the gastric secretory activity. The following table is a typical record from a Pavlov pouch dog, showing the gastric and urinary effects.

TABLE I.
Dog M38. Female, Pavlov pouch, weight 11.0 kilos.

Time	Urine		Gastric Juice		Remarks
	cc.	pH	cc.	Free acid (clinical units)	
9:05	115	5.95			Residual urine
10:05	5	5.96	4.8	23	Control period
(At 10:05, gave 1 mg. of histamine dichloride subcutaneously and 100 cc. water by stomach tube)					
11:05	4	6.95	37.0	115	
12:05	3	7.16	3.5	68	
1:05	4	5.99	5.2	0	
(At 1:05, gave 1 mg. histamine dichloride)					
2:05	5	7.82	39.0	115	
3:05	7	8.02	7.0	102	
4:05	3	5.90	3.5	0	

We believe that our experiments furnish additional evidence that the alkaline tide is due to the formation of HCl in the stomach. While we have found it always present in normal dogs, it seems less pronounced than in the pouch animals, perhaps because of the loss of acid from the fistulae in the latter.

Hiller[3] has reported that histamine lowers plasma pH while raising that of the urine. These observations would seem to indicate that the change in urinary pH after histamine is not brought about by any excess of base, and is perhaps unrelated to gastric secretion. We have confirmed her findings on blood pH following large doses of histamine (1 mg. per kilo or more) but not with such doses as were used in our urine studies (0.06 to 0.08 mg. per kilo). There is following the small doses a rise in blood pH, of 0.02 to 0.06, lasting for 1 to 2 hours.

[1] Ackman, F. D., *Canad. Med. Assn. J.*, 1925, xv, 1099.

[2] Hastings, A. B., and Sendroy, J., *J. Biol. Chem.*, 1924, lxi, 696.

[3] Hiller, Alma, *J. Biol. Chem.*, 1926, lxviii, 833.

3897

The Regeneration of Acid-Fastness by Animal Passage.

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By following up the regeneration of tubercle bacilli reported in a study of the granules of this organism,[1] several strains of non-acid-fast or partially acid-fast organisms have been studied. One or-

ganism or series of organisms obtained from the organs of a patient dying of a generalized miliary tuberculosis will be reported with special emphasis on the ability of the organism to regain acid-fastness on animal passage.

The peculiar aspect of the case was that in the numerous miliary tubercules there were very few acid-fast organisms found. After a search for many hours none were found in the spleen. Instead, however, the miliary tubercles and giant cells contained coccoid bodies (non-acid-fast) arranged singly and in pairs. These possessed a refractile nature not found in ordinary cocci. These cocci, although quite numerous, did not produce colonies on agar media, and sodium hydroxide treatment apparently destroyed their ability to produce infection in guinea pigs.

Pieces of the spleen were placed in celloidin capsules and embedded in the body cavities of guinea pigs. In 3 weeks numerous acid-fast bacilli were found in the capsules. One animal (T93) on which the capsule had broken, had a generalized tuberculosis. The spleen of this animal was inoculated into another animal (T38) that died in 12 days of a septicemia-like condition. The spleen was enlarged many times and red. The lungs were pneumonic, the liver was fatty and acutely inflamed. Numerous coccoid and bacillary organisms were observed as well as organisms that seemed to have a slight acid-fastness. The spleen was macerated, diluted and filtered and a single bacillus colony grown from a picked organism. This organism, a pleomorphic type, that possessed no constant morphology nor cultural characteristics, was inoculated into 2 guinea pigs. One (T78) received about 2 mg. of the organism and died in the same manner at T38 with similar pathology; the other (T82) received about 0.2 mg. and lived to develop a peculiar exudative type of disease resembling tuberculosis, with a few faintly acid-fast organisms in the hilus lymph glands. At this point it was observed that large doses administered in series to other animals produced a rapidly fatal septicemic disease in no way resembling tuberculosis. Small regulated doses produced in the third passage a typical guinea pig tuberculosis.

This process was repeated several more times—once with a single cell strain of a diphtheroid like organism—and the same general result was obtained. The final organism grown on a new special medium resembled the human type tubercle with the exception that it was not quite as virulent for rabbits as a typical human strain.

It is not *B. tuberculosis rodentinum* nor *B. pseudo-tuberculosis.*

According to the postmortem findings in more than 10 patients dying at this institution, a non-acid-fast form of organism has

played the dominant rôle until late in the disease. This is particularly true of the pneumonic phase of the primary complex in infants. Furthermore, it is thought that a great many of the associated conditions in tuberculosis may be attributed to these rapid growing forms that seem to emanate from the tubercle-forming organism in environments not suited for a development of the slowly growing waxy form.

[1] Sweany, H. C., *Amer. Rev. Tuber.*, 1928, xvii, 53.

3898

Nutritional Edema and Its Relation to the Incidence of Common Colds.

FREDERICK HOELZEL. (Introduced by A. J. Carlson.)

From the Department of Physiology, University of Chicago.

In the course of 20 years of personal experimentation with fasting and various diets, edema was frequently manifested. It often occurred after periods of undernutrition but was most prominent after prolonged fasting. Edema has also been observed in others after fasting. It is apparently similar to the starvation edema *(Hungerödem)* which developed among the undernourished masses of Europe during the late war. The European studies of this "war edema" made it clear that nutritional factors and not impairment of the circulation or of kidney function were responsible. But the detailed analysis of the nutritional factors was complicated by the fact that the dietary of the afflicted individuals was not only insufficient in quantity but was also inadequate in other respects. Thus, some investigators were led to attribute the edema mainly to an excessive salt and vegetable intake while others considered it a consequence of deficiency in vitamins or fat.

The edema observed in the subject of the present study occurred independent of some of the factors which complicated the European studies. Hence, it is possible to say definitely that vitamin deficiency, fat starvation or an excessive salt intake were not fundamental factors in the development of this edema. Instead, the observations in this study indicate that protein starvation is the primary factor in giving rise to this type of hydration. The finding of Kohman[1] is hereby supported. However, the gross manifestation of nutritional edema seems to be possible only when the diet con-

tains sufficient salt or carbohydrate or both. Water taken alone is not stored and water restriction only creates thirst without removing the cause of the edema.

Data concerning the state of hydration before, during and after a 33-day fast were secured with the intradermal salt solution test of McClure and Aldrich[2] by Dr. Kunde.[3] Further observations were made later in connection with a 41-day fast. In tests made on the arm and near the knee, the disappearance time of the wheals decreased, by the fifth day after fasting, to about 25% of the pre-fasting rate. At the ankle, it went down to about 5%. With a liberal protein intake following the 33-day fast, the edema practically cleared up within a month; but with 16 days of protein restriction following the 41-day fast, some edema remained for at least 3 months.

A liberal, but not an excessive, protein intake immediately after fasting therefore seemed to mitigate the post-fasting edema but the best results in reducing the severe edema after the 41-day fast were obtained with a few 24-hour fasts and modified fasts. In fact, tests with the method of McClure and Aldrich also showed a dehydration (30 to 40% increase in disappearance time) *during* the 41-day fast. This is contrary to the reports of increased hydration of animals with starvation but the intradermal salt solution test reflects only local (cutaneous or subcutaneous) conditions directly and, very likely, the hydration of starvation is not an edema. The fact that fasting reduces the edema which it helps to create has been a large factor in leading to repeated fasting—now totaling over 500 days.

Nutritional edema, in mild form, seems to be very common. The conditions following illness associated with undernutrition specially favor its development. Hence a consideration of the possible relation of such edema to other common complaints naturally suggests itself. The purpose here is only to emphasize that, in personal experience, a close parallelism between the incidence of colds and nutritional hydration has been observed. Colds were never caught during prolonged fasting or marked and prolonged undernutrition. They developed almost invariably after such periods when edema was also most prominent. Distinct colds (of more than 24 hours duration) have been prevented by keeping the hydration of the organism at a relatively low level, mainly by restricting the carbohydrate intake and maintaining an adequate protein intake. In the light of these observations, colds are regarded as a common consequence of a chilling of highly hydrated and sensitive skin, with the

result that an overload of fluid is thrown upon already hydrated internal structures, including the upper respiratory tract. Bacteria may then play a complicating rôle after excessive secretions have thus been established.

[1] Kohman, E., *Am. J. Physiol.*, 1920, li, 378.
[2] McClure, W. B., and Aldrich, C. A., *J. Am. Med. Assn.*, 1923, lxxxi, 293.
[3] Kunde, M. M., *Arch. Int. Med.*, 1926, xxxviii, 57.

3899

The Penetration of Ultra Violet Light Into the Human Skin.

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The problem of the penetration of ultra violet light into the human skin is not yet solved. This problem is of great interest, however, particularly from the point of view that certain limited parts of the ultra violet spectrum produce certain characteristic biological effects, such as erythema, pigmentation, bactericidy, antirachitic action, and others. For the scientific explanation of this reaction it is of interest to know to what depth of the skin these biologically active rays penetrate and by what substances they are selectively absorbed. In order to contribute to the solution of this problem absorption measurements were made for the various parts and biological constituents of the human skin.

Two methods have been used, the photographic and the photoelectric, both in connection with a mercury quartz arc and a Hilger spectrograph. The photoelectric method has the advantage that the whole spectrum can be obtained within a few minutes, and that it gives a crucial test about the end absorption in the far ultra violet. The photoelectric method gives more exact quantitative results, except at the farthest ultra violet end, where a trace of spectral impurity lowers the exactness of the measurements. Besides, it takes a long time to go over the whole spectrum, and it is difficult to keep the conditions of biologic specimens constant for such a length of time. Both methods used are superior to the thermoelectric method as their pronounced ultra violet selectivity makes them more independent from scattered visible and infrared light.

Results: (1) The difference between living and dead tissue kept

in Ringer solution and on ice for one or two days is smaller than can be exactly measured. (2) The penetration of ultra violet light is stronger than given in the older literature, particularly by Hasselbalch,[1] and smaller than suggested by Macht and his co-workers.[2, 3] (3) The various layers of skin (horny layer, epidermis, corium, sub-cutaneous fat, connective tissue, plasma, blood, melanin, etc.) exert a very different absorption and show characteristic selective absorption bands. (4) The corrected skin sensitivity curve toward erythema, as observed by Hausser and his co-workers[4] can be explained by the passive absorption of ultra violet by the horny layer and the active absorption by the proteins in the skin.

[1] Hasselbalch, K. A., *Strahlentherapie,* 1913, ii, 403.

[2] Macht, D. I., Bell, F. K., Elvers, C. F., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 210.

[3] Macht, D. I., Anderson, W. T., Bell, E. K., *J. Am. Med. Assn.,* 1928, xc, 161.

[4] Hausser, K. W., and Vahle, W., *Strahlentherapie,* 1921, xiii, 41.

3900

Digestive Function in Avitaminoses.

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From the Laboratory of Physiological Chemistry, University of Illinois College of Medicine.

McCarrison[1] and others have observed marked pathological changes in the gastrointestinal tracts of animals kept on diets deficient in vitamins and other substances. From such observations it has been inferred that lesser degrees of vitamine deficiency continued over long periods of time might be responsible for various gastrointestinal disturbances in man. Farmer and Redenbaugh[2] prepared extracts of the pancreas and upper intestines of polyneuritic pigeons and found a decrease in the digestive enzymes as compared with controls.

The present paper represents the first of several attempts to obtain a quantitative index of various gastrointestinal digestive functions in animals and man based on the principle of feeding an excess of difficultly digestible food substances and estimating the percentage digestion by fecal analysis. Iron oxide was used as a key substance to simplify the utilization determinations.[3]

Eight groups of albino rats and 3 groups of guinea pigs were used, each group consisting of 6 animals. Severe conditions of

avitaminosis were induced by feeding diets deficient in vitamins A, B, C and D. The effect of cod liver oil administration and treatment with ultraviolet light was also studied.

As a test of digestive efficiency there was added to the diets in successive periods definite amounts of raw potato starch and elastin or ground horn. The feces were analyzed for starch or protein and for iron and utilization calculated, a correction for metabolic nitrogen being made.

Starch digestion was in the neighborhood of 90% for all animals including controls. For protein digestion values of 51% for animals on -B diet and 60% for rachitic diets as compared with 68% for controls were the greatest variations noted. It is not believed that these differences are great enough to support the view of an early or specific impairment of digestive function in these avitaminoses. Neither did iron reduction tests[4] indicate increased intestinal bacterial activity in these conditions.

[1] McCarrison, R., *J. Am. Med. Assn.*, 1922, lxxviii, 1.
[2] Farmer, C. J., and Redenbaugh, H. E., *Am. J. Physiol.*, 1925-26, lxxv, 45.
[3] Bergeim, O., *J. Biol. Chem.*, 1923, lxx, 29.
[4] Bergeim, O., *J. Biol. Chem.*, 1924, lxii, 45.

3901

Duodenal Drainage of the Human Gall Bladder.

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During the last 5 years both the theory and the efficacy of the Meltzer-Lyon test have been frequently challenged, but as yet no adequate measurements of the amount of bile discharged from the gall bladder following the injection of $MgSO_4$ and other substances into the duodenum, seem to have been made.

In view of the consensus of opinion that $MgSO_4$ is not absorbed by the intestine and believing that evacuation of the gall bladder might be induced by mechanical stimulation, we injected air into the duodenum through a Reyfuss tube and then x-rayed the patient at short intervals—computing the volumes of the gall bladder according to the method employed in previous publications.[1] In 3 out of 4 individuals subjected to this procedure, the gall bladder showed measurable reduction in size after inflation of the duodenum (Fig.

1). In one case (the largest gall bladder we have ever seen) the reduction was considerable, from 96 down to 71 cc. (*A. R.*, Fig. 1). In 2 other cases *(E. I.* and *M. A.)* it amounted to only a few cc., and in the fourth case *(G. A. K.)*, dilation of the gall bladder ensued —a reverse effect which we interpret as a reflex inhibition of the gall bladder, due to sudden and forcible inflation of the duodenum. (*cf.* case *B. B.*, Fig. 1 of accompanying paper).[2]

In each of the 4 cases,egg yolk was subsequently injected into the duodenum in order to test the motility of the gall bladder. It is

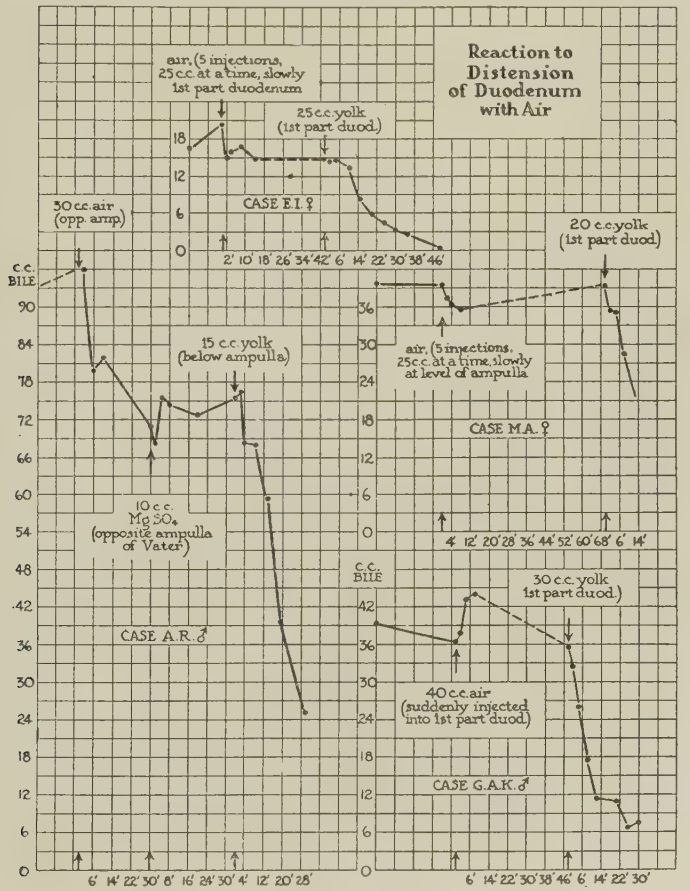

FIG. 1.

Four graphs showing reaction of human gall bladder to inflation of the duodenum through a Reyfuss tube. *cc.*, computed volumes of gall bladder; *2'*, *10'*, etc., minutes after injection of substances into duodenum. Case *E. I.*: epileptic, age 29, wt. 110; case *A. R.*: epileptic, age 16, wt. 165; case *M. A.*: epileptic, age 48, wt. 145; case *G. A. K.*: student, age 30, wt. 130.

interesting to note that when thus administered, this food induces as effective a phase of contraction as when taken by mouth.

Having ascertained the reaction of the gall bladder to inflation of the duodenum, concentrated $MgSO_4$ was then tried. In the first case (*A. R.*, Fig. 2) only a small amount was injected. Due to this, or possibly to the fact that it was administered after the gall bladder had just emptied a third of its contents, the drug was ineffective. In the second case, *(D. S.)* it caused a decrease in volume of about 18 cc. But in the third case (*H. A. S.*, Fig. 2) as much bile was discharged from the distended gall bladder (nearly 40 cc.) as usually occurs during the first phase of contraction following a meal of egg yolk. This was the more noticeable since the gall bladder of this individual reacted but little to previous injections of water and of dilute HCl. A study of the cholecystograms (Fig. 3) shows that within 2 minutes the broad, pear-shaped fundus of the organ markedly narrowed, and that within 15 minutes its volume had diminished from 63 to 25 cc. This rapid change in shape and volume surely indicates that we are dealing with a vigorous contraction of the gall bladder, induced by the presence of $MgSO_4$ in the intestine.

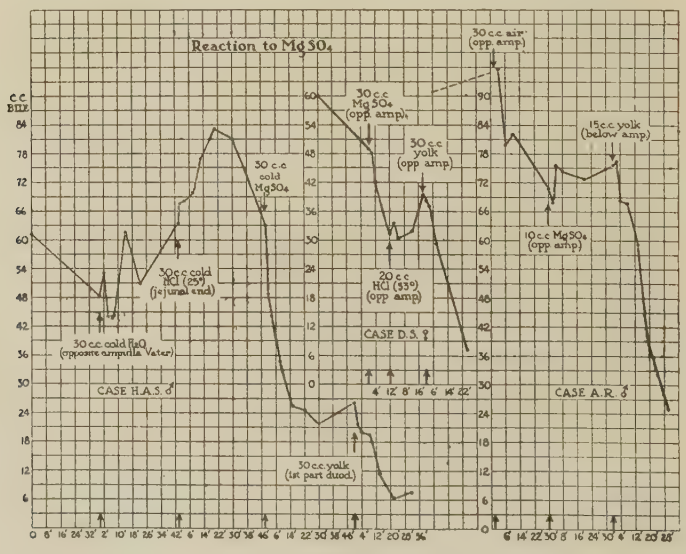

FIG. 2.

Three graphs showing reaction of human gall bladder to duodenal injection of concentrated $MgSO_4$. Case *H. A. S.:* student, age 21, wt. 155; case *D. S.:* epileptic, age 35, wt. 128; case *A. R.:* epileptic, age 16, wt. 165.

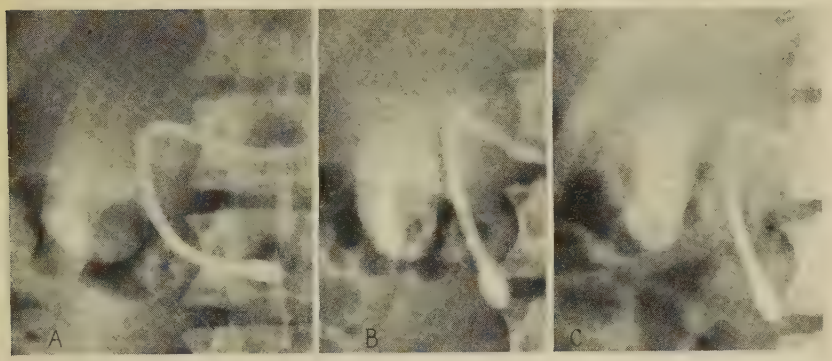

FIG. 3.

Selected cholecystograms showing contraction of human gall bladder after $MgSO_4$ (case *H. A. S.*, Fig. 2). *A*, just before $MgSO_4$: computed volume, 63 cc.; note broad fundus. *B*, 2 minutes afterwards: volume 49 cc.; fundus narrowed, Reyfuss tube forced back by injection. *C*, 15 minutes afterwards: volume 25.5 cc.

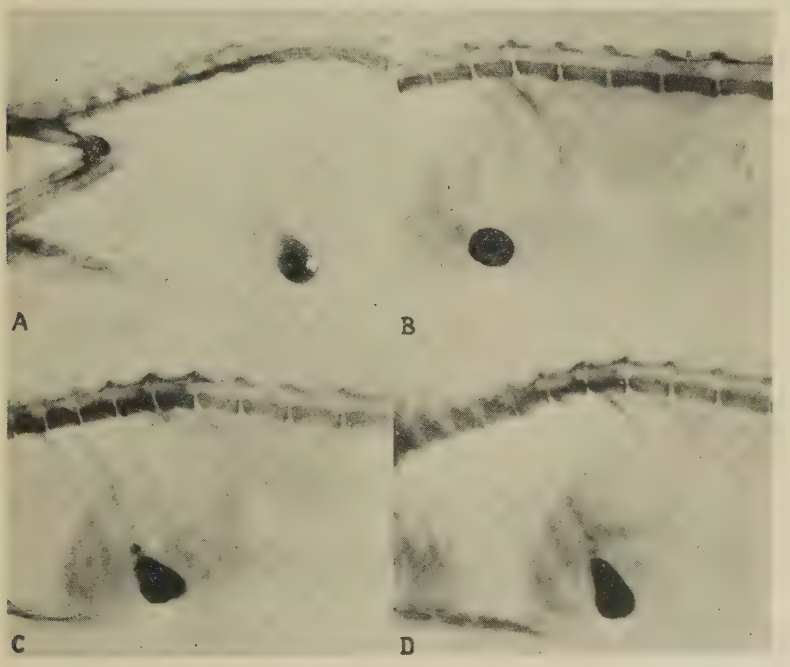

FIG. 4.

Selected X-rays of cat A H., showing reverse effects of injecting $MgSO_4$ and egg yolk into the duodenum after the common bile duct is sectioned. Gall bladder filled with iodized oil. *A*, eight hours after operation. *B*, 3 minutes after injection of a few cc. of concentrated $MgSO_4$; gall bladder relaxed. *C*, 10 minutes after injecting equal amount of egg yolk; gall bladder contracting. *D*, 15 minutes after egg yolk.

At least 3 hypotheses may be offered. *First,* the original one, that the drug paralyzes the smooth muscle of the sphincter papillae. But if it is able to pass through the epithelium of the mucosa it should be absorbed into the circulation and produce a general reaction—a circumstance which rarely occurs. *Second,* that it reacts upon the cells of the mucosa in such a way as to liberate a secretin-like hormone, which activates the musculature of the gall bladder or sphincter. To test the former hypothesis, the common duct of a cat was severed, and the gall bladder filled with iodized oil. Eight hours after the operation, $MgSO_4$ was injected into the duodenum, but the gall bladder failed to respond, although when an equal amount of egg yolk was injected it contracted vigorously (Fig. 4).

The third hypothesis is that the drug stimulates the nerve endings in the intestine, thereby setting up local or spinal reflexes which dilate the sphincter at the end of the common duct. Similarly, the contraction of the gall bladder itself may be explained in 2 ways, either that its musculature is directly activated by a spinal reflex originating in the duodenum, or that the release of pressure in the biliary duct system caused by the opening of the sphincter, stimulates the local nerve net or afferent nerve endings in the wall of the gall bladder, thereby inducing reflex contraction of its tunica muscularis. Whatever the interpretation, there can be no question of the fact that when $MgSO_4$ is injected into the duodenum it induces marked contraction of a distended gall bladder and consequent expulsion of bile.

[1] Boyden, E. A., *Anat. Rec.*, 1926, xxxiii, 201-256.

[2] Boyden, E. A., and Parmacek, L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 462.

3902

Reflex Inhibition of the Human Gall Bladder.

EDWARD A. BOYDEN AND LOUIS PARMACEK.

From the Departments of Anatomy and Pathology, College of Medicine, University of Illinois.

In a previous publication, one of the authors reported that merely drinking a glass of water resulted in a discharge of bile from the gall bladder.[1] In extending these observations to a large number of patients (10 in all) considerable variation was found, ranging from an individual with a discharge of 24 cc. of bladder bile after

a glass of water (case *G. B.*, Fig. 1) to one who reacted not by contraction but by dilation of the gall bladder (case *B. B.*, Fig. 1).

In discussing these extreme types with Dr. W. H. Petersen, the latter suggested that we might be dealing with persons who were differently oriented in regard to the autonomic nervous system. And, indeed, when these individuals were subjected to the Goetsch test, it was found that one extreme *(B. B.) was* markedly vagotonic, registering a decided fall of systolic blood pressure and of pulse rate after subcutaneous injection of adrenalin, and that the other *(G. B.)* was markedly sympaticotonic. No generalizations could be made about the intermediate cases.

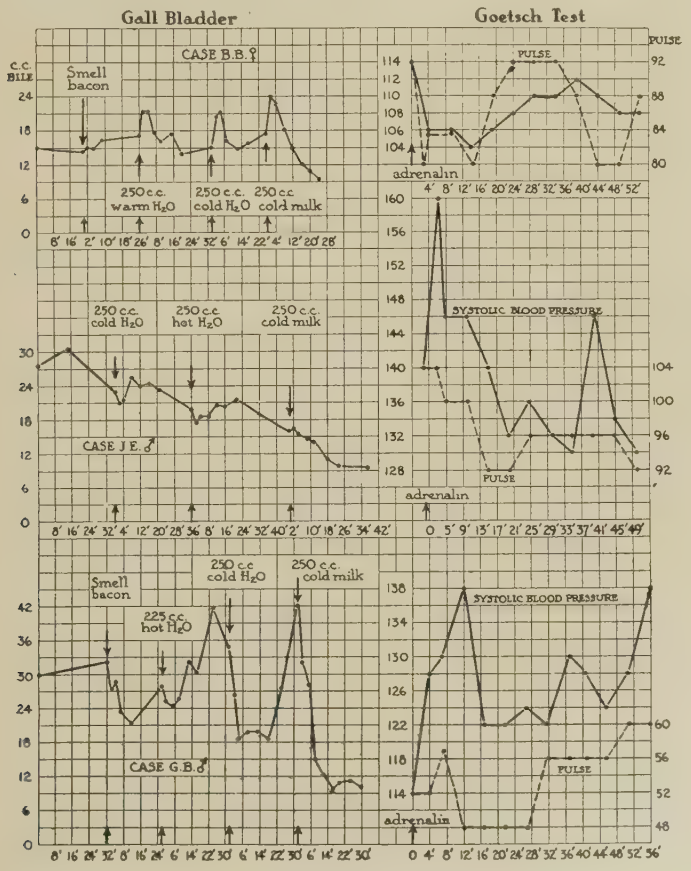

FIG. 1.

Selected graphs showing 3 types of reaction induced by drinking a glass of water. The 3 curves on the left record the changing volumes of the gall bladder; those on the right, the systolic pressure and pulse rate after subcutaneous injection of 0.5 cc. of adrenalin (1:1000). Case *B. B.*, student, age 19, wt. 115; case *J. E.*, student, age 21, wt. 130; case *G. B.*, technician, age 60, wt. 155.

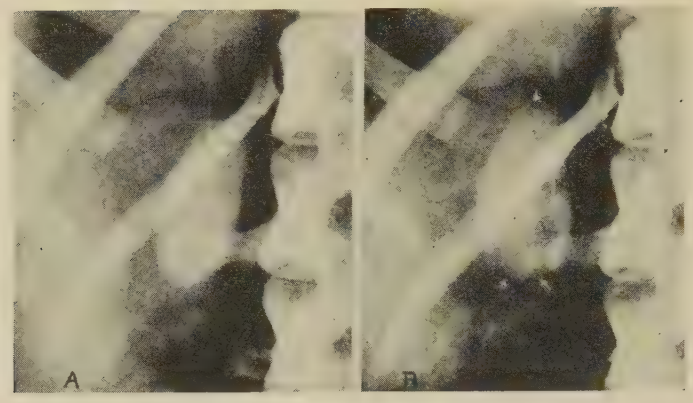

FIG. 2.

Selected cholecystograms (case *B. B.*). *A*, immediately before drinking glass of cold water; *B*, two minutes later. *Cross*, projected center of target engraved upon x-ray table.

Up to the present writing we have not been able to secure a gall bladder record of another case showing so marked a fall in pulse rate. But whether or not this reaction of the gall bladder proves to be characteristic of vagotonics, the present study is of value in that it has revealed a reversed type of response. In the individual in question (*B. B.*, Fig. 1) we secured sudden dilation of the gall bladder, after giving the patient a glass of warm water, of cold water and of milk. That this is not merely a filling of the reservoir following abrupt closure of the sphincter but is due to relaxation of the gall bladder musculature, is indicated by 2 observations: first, the rapidity with which the change takes place (see cholecystograms, Fig. 2); and second, the contrast which it presents to case *J. E.* (Fig. 1) in which there is a slow filling of the gall bladder after each response to water, without marked relaxation of its muscular tonus—a case which is probably characterized by so hypertonic a sphincter that even the contraction of the gall bladder after milk is relatively ineffective.

A similar case of dilation induced by forcible inflation of the duodenum through a Reyfuss tube, is presented in an accompanying paper (case *G. A. K.*, Fig. 1).[2] These 2 cases suggest that the gall bladder is under nervous as well as hormonal control. For we are unable to explain its rapid dilation in any other way than that of reflex inhibition of its musculature.

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[1] Boyden, E. A., PROC. SOC. EXP. BIOL. AND MED., 1927, XXV, 99.

[2] Boyden, E. A., and Saunders, A. M., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 458.

Iowa Branch.

University of Iowa, February 1, 1928.

3903

Mineral Content of Saliva of Children with Arrested Dental Caries.

MIRIAM ROSKIN. (Introduced by J. D. Boyd.)

From the Department of Pediatrics, State University of Iowa.

Numerous instances of arrested dental caries in childhood have been noted in the routine dental examination of patients in the Children's Hospital during the past 2 years. Such arrest of caries in childhood is decidedly unusual. Correlation of dental findings with hospital records revealed that without exception, each of these children exhibiting arrested caries had diabetes mellitus, and was under strict dietary control. It was further noted that the incidence of salivary calculus was much higher in these patients than in the other patients examined.

The present study was undertaken in order to determine whether or not this arrest of dental caries was associated with alterations in the mineral content of the saliva. One group studied consisted of 17 diabetic children, 15 of whom had been under dietary control for a year or more; the other of 13 non-diabetic patients of similar age. The incidence of dental caries in the non-diabetic group was very high. Of the diabetic children, active caries was noted only in 2 cases whose diabetes was of very recent origin, and in a third child who has not been under satisfactory dietary control.

Whole saliva was collected before breakfast, without stimulation of the glands and without water drinking. The chloride content was determined by Van Slyke's method, pH by the method of Clark and Lubs. Calcium and phosphorus were determined on ashed saliva, following the technique of Clark and Levine. The analytical results are summarized in the accompanying table. A high phosphorus value was frequently, but not invariably, accompanied by a high calcium value. When unusual values for calcium and phos-

phorus were found in saliva, blood determinations were likewise made, but in no case were abnormal findings noted.

SUMMARY OF SALIVARY ANALYSIS.

	Diabetic	Non-diabetic		Diabetic	Non-diabetic
	mg.%	mg.%		mg.%	mg.%
Calcium			Chlorine		
High	7.75	7.73	High	90.8	83.0
Low	3.7	2.8	Low	47.4	30.6
Mean	5.7	5.5	Mean	69.6	55.0
Phosphorus			pH		
High	17.8	16.6	High	7.0	7.3
Low	8.7	7.1	Low	6.3	6.3
Mean	12.1	11.5	Mean	6.8	6.9

The wide individual variations in mineral content of saliva noted in this study were also observed by Clark and Levine[1] in the saliva of adults, although the phosphorus values obtained by them appear to average slightly higher than those noted in this series.

Summary: The mineral content of saliva was studied in 2 groups of patients; one of diabetic children having resistant teeth and arrested caries, the other of unselected hospital patients showing as a group marked caries and soft teeth. The variations noted in the calcium, phosphorus, chlorides and pH of the saliva were similar in both groups, indicating that the formation of salivary calculus, and the arrest of dental caries noted in the diabetic children under dietary control, are probably not due primarily to any marked differences in the mineral content of the saliva.

[1] Clark and Levine, *Am. J. Physiol.*, 1927, lxxxi, 264.

3904

Relief of Parathyroid Tetany by Injections of Uranium Nitrate.

W. W. SWINGLE.

From the Zoological Laboratory, State University of Iowa, Iowa City.

Recent studies from this laboratory[1, 2] have shown that slight changes in the acid-base equilibrium, with a shift in the reaction of the blood toward the acid side, exert a profound effect upon the tetany of parathyroid insufficiency.

The writer and his collaborators have shown that asphyxiation by CO_2 of dogs presenting violent tetany promptly brings about

a return to normal in so far as tetany symptoms are concerned. The disappearance of symptoms was correlated with disturbance of the acid-base equilibrium, *i. e.,* lowered CO_2 capacity, CO_2 content and pH, due to rise in lactic acid. Tetany again supervened following a return to a normal acid-base balance and disappearance of the lactic acid. The total serum calcium remained unchanged by the CO_2 treatment, despite the fact the animals seemed normal and were free from tetany.

Since the effect of the CO_2 lasts but a brief period, and since it was considered desirable to study the experimental animals over longer periods, the present experiment with uranium nitrate was undertaken. This substance has been extensively employed in the study of renal function since it induces marked pathological changes in the kidneys when injected, and it is a well established fact that a profound acid intoxication develops in animals so treated. It is not definitely known to what acid or acids the acidosis of uranium nitrate poisoning is due. The intoxication develops slowly and reaches a maximum between the 4th and 8th day following injection.

In the present experiments both old and young dogs were used. They were thyroparathyroidectomized and allowed to develop marked tetany symptoms. They were then bled for CO_2 capacity, pH and serum calcium. It has been the experience of those working in this laboratory[3] that the CO_2 capacity, and pH of tetany dogs are normal. After bleeding, the tetany animals were injected with uranium nitrate subcutaneously. The dosage depended upon the age of the dog, for, as Mac Nider has emphasized, old dogs are much more susceptible to uranium nitrate than are younger animals. However, none of the dogs received more than 6 mg. per kilo.

The effect of uranium nitrate upon tetany is indeed striking, for within 12-24 hours after injection, all tetany symptoms have disappeared and the animals run about, eat and play in normal fashion. The normal condition may last a week or 10 days (dogs fed heavy meat diet) before tetany again develops. Examination of the blood of the injected dogs show a lowered CO_2 capacity, CO_2 content and a slightly lowered pH. The serum calcium may remain unchanged or increase somewhat. The increase is seldom more than 1 mg. per 100 cc. of blood. In general the serum calcium remains low. However, despite the low calcium content of the blood the dogs appear normal. Table I summarizes the data obtained from study of 2 typical cases.

The answer to the question why changing the reaction of the blood slightly toward the acid side brings about cessation of tetany

TABLE I. *Effect of uranium nitrate on parathyroid tetany.*

Dog	Operated	Tetany	Bled	CO_2 Capacity	pH	Ca	Remarks
4	Feb. 6, '28	Feb. 10	Feb. 10	38.9	7.34	7	Marked tetany. Injected (6 mg. per kilo) uranium nitrate 10 A. M. Feb. 10 complete recovery from tetany after 24 hrs. Dog placed on heavy meat diet.
			Feb. 12	37.3	7.36	8.2	Seems normal.
			Feb. 20	28.3	7.34	8	Animal normal.
7	Feb. 11, '28	Feb. 13	Feb. 13	47.1	7.39	8	Animal in tetany—given 5 mg. uranium nitrate per kilo. Normal after 36 hrs. Eats meat and runs about.
			Feb. 15	40.9	7.35	7.1	No tetany symptoms. Heavy meat diet.
			Feb. 18	32.4	7.35	5.8	No tetany. Heavy meat diet.
		Feb. 21	Feb. 20	36.8	7.39	5	Tetany present. Fine muscular tremors and jerking. Refuses food. Given 3 mg. uranium nitrate per kilo.
			Feb. 22	30.2	7.25	7	Tetany symptoms disappeared after 24 hrs. In coma when bled. Died. Uranium poisoning.

symptoms and small increases in the serum calcium is not forthcoming at the present state of our knowledge of the pathogenesis of tetany. It seems probable, however, that disturbance of the acid-base equilibrium with a shift in reaction toward the acid side, relieves tetany by rendering the serum calcium more diffusible, and also probably takes care of any excess of phosphorus which may be present by stimulating its excretion.

[1] Swingle, W. W., Wenner, W. F., and Stanley, P., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 165.

[2] Wenner, W. F., *Am. J. Physiol.*, 1927, lxxxi, 612.

[3] Wenner, W. F., and Muntwyler, E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 480.

3905

Castration and Ovariectomy on Spontaneous Activity and Ability to Learn.*

W. W. TUTTLE AND S. DYKSHORN.

From the Departments of Physiology and Zoology, State University of Iowa.

The present investigation is directed toward the establishment of the relationship between spontaneous activity and the ability to learn, with special reference to castration and ovariectomy, on these processes. This is a preliminary report of the first experiment undertaken to establish the relationship mentioned above.

Data were collected from 5 litters containing 35 rats under 50 days of age. The ability of each rat to learn was measured by means of a maze described by Rickey.[1] Spontaneous activity was determined by the revolving cage method described by Durrant.[2] Fifteen animals were operated in an attempt to modify their activity, while 20 were used as controls. Data were collected with reference to the number of trials required to learn the maze and the number of errors made in so doing. The learning time was recorded in seconds and the spontaneous activity in number of revolutions. Table I shows the data collected up to the present time. The figures presented under "activity" represent the average daily number of revolutions run over a period of 10 days.

The data in Table I reveal the fact that in case of litters 1 and 2, castration had no effect on either the learning process or spontaneous activity. They show also that in litter 4, the castrates had a distinct advantage over the normals in all parts of the experiment. In litters 3 and 5, just the reverse is true since the normals have the advantage.

TABLE I.

Litter	No. trials		No. errors		Time in sec.		Activity	
	Controls	Castrates	Controls	Castrates	Controls	Castrates	Controls	Castrates
1	18	20	40	35	484	478	2376	2191
2	15	15	67	53	741	748	2534	2586
3	14	14	43	61	360	556	2941	1952
4	25	11	52	30	436	273	2415	2935
5	11	15	29	45	347	473	2039	1232

* The expenses of this investigation were defrayed by a grant from the committee on research in problems of sex of the National Research Council. The experiments were conducted in the Zoological Laboratory.

Although the data in Table I indicate that there is a relationship between spontaneous activity and learning, this point is shown to a better advantage in Table II. Here, in each litter the individual rats are ranked according to their speed in learning the maze. Opposite the learning rank is the rank of activity. For example, the rat in litter 1 which ranks first in learning is second in activity.

TABLE II.

Litter 1		Litter 2		Litter 3		Litter 4		Litter 5	
Learning	Activity	Learning	Activity	Learning	Activity	Learning	Activity	Learning	Activity
1	2	1	1	1	1	1	1	1	1
2	1	2	4	2	3	2	4	2	2
3	7	3	3	3	5	3	5	3	5
4	3	4	5	4	4	4	3	4	4
5	5	5	2	5	2	5	2	5	3
6	4	6	6			6	7	6	7
7	6					7	6	7	6

The data in Table II show that in every case, except litter 2, the animal which was the most adept in maze learning was the most active animal in his litter. Furthermore, it is seen that the animals which learned slowest were the less active, except in litter 3. In the intermediate groups 5 animals fall in the same rank for both activity and learning. The arrangement of the rest of the data is such that the relationship between activity and learning is not well defined.

At the present time the data indicate that castration has no effect upon either the learning or the activity of white rats under the age of puberty. This seems to be in accord with previous investigations. Furthermore, it seems that activity and learning are closely allied, since the most active animals learn fastest, and the least active slowest.

[1] Rickey, Edna, Dissertation, Ohio State University, 1-76.

[2] Durrant, E. P., *Am. J. Physiol.*, 1924, lxx, 344.

3906

I. Effect of Placental Extract on Mammary Glands of Male Guinea Pigs.*

H. O. HATERIUS. (Introduced by W. W. Swingle.)

From the Zoological Laboratory, University of Iowa.

Although considerable work has been centered upon the influence of placental hormone upon secondary sexual characters in the female, particularly the rabbit and the guinea pig, little attention has been directed toward its possible effects upon those of the male. Hermann[1] states briefly that he induced development of the mammary glands in the young male rabbit, through injection of the "active substance" of corpus luteum and of placenta, even to the extent of producing milk secretion, and he concludes that this activating substance is of powerful influence in the formation of specific sex characters. No evidence is offered, however, in support of his statements, nor are any particulars of the experiments given. Fellner[2] reports in somewhat more detail 2 cases of a similar nature in young rabbits, in which a slight degree of hypertrophy was induced but, unlike Herrmann, he was unable to produce a secretion of milk.

In connection with some work with the placental hormone, 4 adult guinea pigs were castrated and injected twice daily with ½ cc. doses of human placental extract (furnished through the courtesy of Parke Davis & Co., Detroit, under the trade name of "Estrogen"): the total daily dosage amounting to 20-25 rat units. Three control castrates were given equal amounts of normal saline.

After 8 days of extract injection, a swelling of the mammary regions became noticeable; the nipples increased in size and the surrounding areolar areas assumed a turgescent appearance. This hypertrophy continued steadily over a period of some 3 weeks, after which time continued injections produced no further effect. The hypertrophied glands, together with controls, were removed, photographed *in toto,* and sections were made of nipples and of glandular tissue. In the injected animals the nipples displayed a marked hypertrophy, evidenced particularly by the epithelium and by a thickening of the stratum corneum, there being an increase to approximately 3 times that of the controls. The glandular structures, extremely small and scanty in the untreated male, increased in proportion through a decided growth and branching of ducts, through growth of the alveoli, and through widening of the lumina. This

* The expenses of this investigation have been defrayed by a grant from the Committee on Research in Problems of Sex of the National Research Council.

held true also for the lobules, although there appeared to be no increase in their actual number. There was evidence of active secretion as indicated by the presence of colostrum corpuscles and what appears to be milk.

[1] Herrmann, E., *Monatsschr. f. Geburtsh. u. Gynak.*, 1915, xli. 1.
[2] Fellner, O., *Arch. f. Gynak.*, 1913, c, 641.

3907

Effect of Bilateral Nephrectomy Upon the Acid-Base Equilibrium of Dogs.*

W. W. SWINGLE, W. F. WENNER AND P. STANLEY.

From the Zoological Laboratroy, State University of Iowa.

For several years the senior writer and his students have been studying the effect of bilateral adrenal extirpation in cats and dogs and reached the conclusion that one of the train of causes resulting in death from adrenal ablation is acid intoxication.[1, 2, 3, 4] As a result of our experiments the hypothesis was advanced that the adrenal cortex secretes a hormone which in some manner assists in maintaining the normal functioning of the kidney. We were interested in the fact that the type of acidosis which appears during adrenal insufficiency is similar to that occurring in uremia. As a further means of testing the idea whether or not the kidney is involved in adrenal insufficiency the present writers undertook to make a careful comparison of the symptoms and blood findings occurring in adrenal insufficiency with those which follow kidney extirpation.

Large, well nourished dogs were employed for the kidney work —the average weight being 18-20 kilos. The right kidney was extirpated and after a 7 to 10 day interval the left kidney was removed. Animals so operated generally remain normal for several days before untoward symptoms develop. The unilaterally nephrectomized dogs were bled for CO_2 capacity, CO_2 content, pH, phosphorus, sulfur, chlorides, sugar and urea. Later when symptoms of renal insufficiency appeared the animals were bled at various times.

The first symptoms noted were anorexia and lassitude, the animals appearing normal otherwise. Later they vomited considerably, refused all food and had to be fed daily 200-400 cc. of milk by stomach tube. Weakness of the hind limbs appeared, drowsiness

* Part of the expenses of this investigation were defrayed by a grant from the Bache fund of the Natural Academy of Science.

and coma. Our animals survived the double operation for varying periods (3-6 days). It is probable that the survival period would have been greater if the animals had not been bled repeatedly.

The results show that acid intoxication is not a prominent symptom of renal insufficiency in dogs—in fact our data indicate that marked acid intoxication does not appear in nephrectomized dogs even near the terminal stages. There is usually a slight drop in the CO_2 capacity and content, a negligible change in pH, marked increase in inorganic sulfate and phosphate and urea, some increase in blood sugar and a marked drop in chloride. We are not prepared to say at this time, whether or not the quantity of chloride lost in the vomitus is sufficient to offset the marked increase in inorganic phosphate and sulfate, thereby maintaining an approximately normal acid-base equilibrium.

There are some striking resemblances and also marked differences between the blood picture of adrenalectomized and nephrectomized dogs. Adrenalectomized animals invariably develop a marked uncompensated acidosis with low CO_2 capacity, CO_2 content, pH, and sugar. The blood sugar of such animals falls despite forced feeding, in the later stages of adrenal insufficiency. The amount of urea, non protein nitrogen, inorganic phosphate and sulfate in the blood are greately increased in both nephrectomized and adrenalectomized animals. All dogs survived the second operation approximately the same length of time, *e. g.,* 4-6 days. It will be noted at once that the chief difference between nephrectomized and adrenalectomized dogs are: Adrenalectomized dogs with intact kidneys show marked acid intoxication and fall in blood sugar, nephrectomized dogs, on the other hand, show but slight evidence of acidosis and their blood sugar rises and remains higher than normal up until the time of death. Moreover, phosphate and sulfate values are considerably higher in the nephrectomized dogs showing symptoms than in adrenalectomized animals—but despite this increase acid intoxication does not appear as a marked symptom.

It would seem not improbable, therefore, that the acidosis of adrenal insufficiency and probably also of uremia is extrarenal in origin and probably only partially dependent upon kidney injury because, as we have indicated, complete kidney removal does not produce such symptoms or at most, but very slight symptoms.

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[1] Swingle, W. W., and Eisenman, A., *Am. J. Physiol.,* 1927, lxxix.

[2] Swingle, W. W., and Wenner, W. F., PROC. SOC. EXP. BIOL. AND MED., 1927, XXV, 169.

[3] Swingle, W. W., *Am. Naturalist,* 1927, lxi, 132.

[4] Yonkman, F., PROC. SOC. EXP. BIOL. AND MED., 1927, XXIV, 786.

New York Meeting.

University and Bellevue Medical College, March 21, 1928.

3908

Sex and Seasonal Differences in Weight of Liver and Spleen.

OSCAR RIDDLE.

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Neither sex nor seasonal differences in the weight of either liver or spleen seem to have been established hitherto in animals. Both of these relationships are demonstrated in ring doves *(Streptopelia risoria)* by the data submitted here. In the human it is considered that the weight of the male liver is roughly 50 to 60 ounces; that of the female, 40 to 50 ounces (Cunningham). This difference is apparently roughly proportional to sex differences in body weight. Adequate and comparable data from healthy humans are obviously not available. It is suggested that earlier failure to find sex differences[1] in the albino rat is due either to insufficient numbers of weights from adults, or, to the obliteration of differences by caging. The rats used for those measurements had probably been more closely confined than were the doves of the present study. In the livers from approximately 50 male and 50 female Leghorn fowls, killed from hatching to maturity by Latimer,[2] "there seems to be a tendency for the females (livers) to run higher (possibly 1% higher) than in the males. This is reversed for the 6 older chickens." Present data from fowls are inconclusive and evidently inadequate.

All the animals used in our calculations were free from obvious disease, and were sexually mature (6-30 mo.). All were killed by decapitation and the glands weighed at once. The weights were obtained during all months of the year. January, February and March constitute "winter"; April, May, June, "spring"; July, August, September, "summer"; October, November and December form the "autumn" period. The data obtained from 19 races or strains

were first plotted separately for sex and season. The mean for these 19 races is the figure used in Table I.

TABLE I.
Seasonal size of liver and spleen in healthy male and female ring doves.
(Means for 19 races.)

Seasons	No. birds	Age (mo.)	Weights (grams) of			
			Body	Gonads	Liver	Spleen
			Males.			
Winter	128	15.7	160	1.022	2.870	.037
Spring	142	14.6	158	1.225	2.956	.039
Summer	98	17.6	155	1.142	3.169	.042
Autumn	131	17.9	160	0.912	3.047	.037
			Females.			
Winter	129	15.6	158	.345	3.146	.046
Spring	112	14.8	155	.363	3.402	.053
Summer	83	15.5	153	.328	3.342	.048
Autumn	120	17.7	157	.307	3.291	.044

Sex difference. Female livers are heavier than those of the males of the corresponding season in all cases. They average 9.4% larger, despite the fact that the body weight of the males is greater by nearly 2%. The difference is not ascribable to age; the mean age of the males being 16.4 mo. and that of the females 15.9 mo.

The weight of the female spleens exceeds that of the males by an average of 23.5%. The female spleens are the larger at all seasons.

Seasonal difference. The livers of both males and females are smallest during autumn and winter; largest during spring and summer. The spleens follow precisely the same rule. In both sexes the body weight is slightly reduced at the period of largest size of spleen and liver. The male liver averages 10.4% larger in summer than in winter; the spleen, 12.0% larger. The female liver is 6.1% larger in summer than in winter; the spleen, 4.3% larger. There is thus some evidence that the seasonal fluctuation of size in liver and spleen is greater in the male than in the female.

The size of the gonads is also tabulated; with one exception among the females the figures show, as do those obtained from still other kinds of pigeons earlier described,[3] that the ovaries and testes are largest in spring and summer—the period of smallest thyroid size. It is thus now shown that the spleen, liver, ovary and testis are largest at the season the thyroids are smallest. For the first time in any animal this association of size changes has been established for these several organs.

Summary: (1) Weights of liver and spleen obtained at all months of the year on 499 male and 444 females, healthy, adult ring doves demonstrate that a true sex difference exists. Though the male body weight is slightly larger, the male livers and spleens are smaller, 9.4%, and 23.5%, respectively. (2) A true seasonal increase in size of liver and spleen occurs in spring and summer in both sexes (10.4% and 12.0% in ♂♂; 6.1% and 4.3% in ♀♀). (3) These changes in spleen and liver are positively correlated with size changes in testis and ovary; and negatively correlated with size changes in the thyroids of these animals.

[1] Donaldson, H. H., *The Rat*, Philadelphia, 1924.
[2] Latimer, H. B., *J. Agr. Res.*, 1924, xxix, 363-397.
[3] Riddle, O., *Am. J. Physiol.*, 1925, lxxiii, 5-16.

3909

Significance of Female Sex Hormone Reaction in the Male Blood.

ROBERT T. FRANK AND M. A. GOLDBERGER.

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In 1925, simultaneously and independently, Loewe of Dorpat[1] and one of us with collaborators[2] demonstrated the presence of the female hormone in the circulating blood of females by means of the rodent vaginal spread test.[3] Since then, in numerous publications we have attempted to simplify and standardize the method of extracting and testing human blood for the female sex hormone.[4] Among other applications we advocated the use of this test to determine the sex of pseudo-hermaphroditic individuals in whom we regarded a positive reaction, appearing cyclically, as a proof of the presence of functioning ovaries and feminine sex.[5] Our preliminary work had shown that large quantities of bull's blood (150-100 cc.) gave a negative reaction when extracted by our method. The same applied to concentrated lipoid, HCl, saline and watery extracts of bull's testes, as well as extracts of the hypophysis, thyroid and adrenal, liver, muscle, various proteins, etc.[6] The work of Dohrn,[7] who claimed to have obtained a positive reaction with male urine first called our attention to the possible non-specificity of the Allen and Doisy reaction. After our investigation on male bloods had been completed, the short article of Hirsch,[8] who used our method,

appeared. In the 4 male bloods which he examined, he has found a positive reaction.

To date we have obtained 70 bloods from 55 males. Of these, 10 had to be discarded because the injected mice died early. The technic was that mentioned in our last article,[4] in which 40 cc. of blood were dried with sodium sulphate, extracted with ether, the dry ethereal extract taken up in 2 cc. of water and injected. Our readings are: 0 to —2 = no reaction; 2 to 2+ = weak reaction; —3 to 3 = threshold reaction; 3 to 4 = strong reaction.

Our results are, therefore, based on 60 bloods from 47 patients. Of these, 4 showed a weak reaction (2 to 2+) 3 showed a threshold reaction (—3 to 3), 40 showed a negative reaction.

Two of the individuals investigated were healthy young males from whom weekly, over a period of four weeks, 80 cc. of blood was obtained, permitting us to run a double test (40 cc.). No cyclical reaction was noted, although one test gave a 2, the second sample obtained on the same day reading 0. Neither the age or the fertility of the positive subjects appears to play any rôle. Age 19-22-24-25-45-53-55 years. Children 0-0-0-0-3-5-3.

We extracted 12 urines obtained from males, injecting the concentrates into test mice. No positive results were obtained even with total quantities of 450 to 570 cc., although 5 cc. of urine from pregnant women similarly extracted gave positive results.

We tested the effect of the female sex hormone on capons by injecting a lipoid solution containing 2 rat units daily for 40 days and 5 rat units for 8 days without noticeable effect on the comb, wattles, spurs, or behavior.

The findings may be variously interpreted: (1) The vaginal spread reaction may not be specific. (2) The male and female sex hormone may produce the same reaction in the castrate rodent. (3) Certain males, though apparently normal, may be latent hermaphrodites (ovotestis).

The question naturally arises as to whether these findings in the male in any way diminish the applicability or nullify the interpretations of our findings obained in females. In the hundreds of blood tests we have made, our results have been amply confirmed by the clinical course after prolonged observation or after operation.. The occasional and as yet unexplained positive results in males do not reduce the value of our test, even as much as the occasional positive outcome of the Wassermann reaction in a non-syphilitic renders this widely used test less valuable.

We desire, however, to caution against the use of our blood test

in the determination of sex[5] until further study assures us that the *cyclical* appearance of a positive reaction is unquestionably limited to females.

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[2] Frank, R. T., Frank, M. L., Gustavson, R. G., and Weyerts, W. W., *J. Am. Med. Assn.*, 1925, lxxxv, 510.

[3] Allen, E., and Doisy, E. A., *J. Am. Med. Assn.*, 1923, lxxxi, 819.

[4] Frank, R. T., and Goldberger, M. A., *J. Am. Med. Assn.*, 1926, lxxxvii, 1719; *J. Am. Med. Assn.*, 1928, xc, 106; *J. Am. Med. Assn.*, 1928, (February 4).

[5] Frank, R. T., and Goldberger, M. A., *J. Am. Med. Assn.*, 1926, lxxxvii, 554.

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[7] Dohrn, M., *Klin. Wchnschr.*, 1927, vi, 359.

[8] Hirsch, *Klin. Wchnschr.*, 1928, vii, 313.

3910

Immunity in Guinea Pigs to the Virus of Vesicular Stomatitis.

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It is known that the injection of immune serum into guinea pigs prevents generalization of the lesions but not the primary vesicles of foot-and-mouth disease. In studying a strain of the virus of vesicular stomatitis, a disease of horses closely related to foot-and-mouth disease of cattle,[1] we have found that the virus, when injected into guinea pigs, loses its original feeble power to produce the characteristic secondary lesions in the pad, and that only primary lesions arise after pad inoculation. Notwithstanding this fact, the virus receives a general distribution since it can be recovered, 48 hours after pad inoculation into guinea pigs, from the apparently normal tongue. On the other hand, when the virus is injected into the muscles or the skin (intradermal) elsewhere than in the pad, no local lesion whatever follows, and 10 days after the inoculation it is found that the pigs are immune to reinoculation.

In the preliminary experiments, no attempt was made to titrate the strength of the virus, because present methods are crude, and once the infectivity of a given sample of virus has been determined, it is impossible to estimate the rate of its deterioration. Guinea pig pad vesicle fluid, obtained 24 to 48 hours after inoculation, diluted 1:10 and 1:20 with phosphate buffer at a pH of 7.5; and filtered

through a Berkefeld "V" candle, was employed in the following experiments. The immune serum was obtained from guinea pigs 10 to 14 days after inoculation.

Neutralization of the virus *in vitro* was first undertaken. 1 cc. of a 1:10 dilution of the virus was added to 1 cc. of immune serum and the contents of the tubes thoroughly mixed. The tubes were kept at room temperature for one minute, one hour, and 24 hours respectively, at which times the virus-serum mixture was injected into the pads of normal guinea pigs. Normal guinea pig serum was used as a control. Lesions appeared only in the animals treated with normal serum. After 10 days the pigs inoculated with immune serum and virus were retested by pad inoculation. All developed typical lesions.

Next, the pads of 4 normal guinea pigs were infiltrated with immune serum and the pads of 2 with normal guinea pig serum. One hour later the pads of 2 of the guinea pigs treated with immune serum, and those of one pig with normal serum were injected with a 1:20 dilution of the virus. The remaining 3 pigs were similarly inoculated with a 1:20 dilution of the virus on the following day. Lesions appeared only in the pads infiltrated with normal serum. Ten days later, all animals were reinoculated in the pads with active virus, and only the immune serum group developed typical vesicles.

Following this experiment, we determined the protection given by the intramuscular injection of immune serum, succeeded by intracutaneous or intramuscular inoculation of the virus. 0.5 cc. of immune serum was injected into the right thigh muscles of 8 normal guinea pigs. One hour later 2 animals were injected in the pads with a 1:10 dilution, and 2 with 0.5 cc. of a 1:20 dilution of the virus in the left thigh muscles; 24 hours later this procedure was repeated with the 4 remaining guinea pigs. Typical lesions appeared in the pads of the 4 animals inoculated in this tissue, while those receiving the virus intramuscularly revealed no manifest lesions. On retesting 10 days later with active virus, the animals inoculated intramuscularly developed typical lesions.

The conclusions which we draw from these experiments are. (1) that the virus generalizes through the body, although it induces no visible changes; (2) that following single intramuscular or intracutaneous inoculation of living virus at sites other than the pads, although no manifest lesions occur, a solid immunity results; (3) that a certain degree of neutralization of vesicular stomatitis virus *in vitro* and *in vivo* results from the addition of the serum of im-

mune animals; (4) that no immunity results from the injection of neutralized virus.

[1] Olitsky, P. K., Traum, J., and Schoening, H. W., *J. Am. Vet. Med. Assn.*, 1926, lxx, 147; Olitsky, P. K., *J. Exp. Med.*, 1927, xlv, 969.

3911

Mechanism of the Inhibition of Bacteriophagy by Agar or Gelatin.

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It has been stated that an increase in the concentration of gelatin or agar in the medium tends to inhibit the lysis of bacteria by bacteriophage. d'Herelle explains this action on the assumption that the excess of gelatin or agar inhibits the normal growth of *Bacteriophagum intestinale* by interfering with the free diffusion of the products of its metabolism.[1] This explanation is not acceptable so long as there exists no satisfactory evidence of the metabolic activity of bacteriophage. Our recent studies have shown that lysis of bacteria may be the direct result of rupture of the bacterial cells due to increased uptake of water from the medium.[2] If this is true, inhibition of lysis in the presence of high concentrations of agar or gelatin in the medium may result from a competition for water between the medium and the bacteria.

Petri plates containing nutrient medium of different concentrations of agar or gelatin were seeded with susceptible bacteria and subsequently minute droplets of bacteriophage were deposited at different places on the seeded surface. The plates were allowed to dry for one hour under porous clay covers both before and after deposition of phage, in order to prevent its spreading. Contact impressions were taken at regular intervals on coverslips from the spots on which phage was deposited.

Macroscopic observation of the plates showed that lysis of bacteria occurred only in the plates containing low concentrations of agar or gelatin (1 to 2% and 15 to 25% respectively). The plates containing 4% agar, as well as those containing 50% gelatin, showed, on the contrary, a marked increase in the density of bacterial growth on the spots where phage was deposited, from which it was concluded that phage exerted a stimulating effect on the

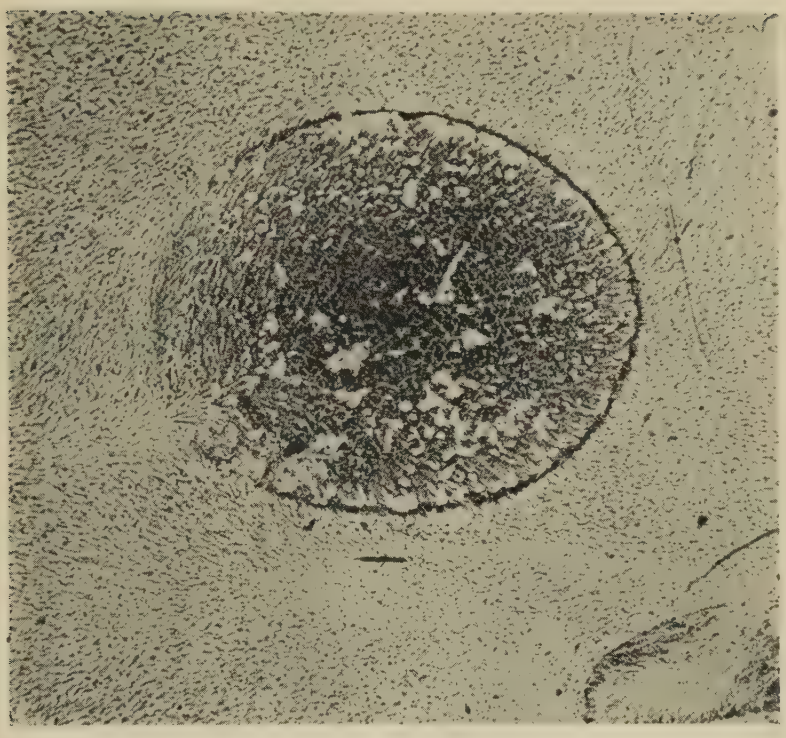

FIG. 1.

growth without causing lysis. (See Fig. 1.) Microscopic examination of stained contact impressions showed that previous to lysis, the bacteria underwent marked swelling on the media of lower concentration, while on the media of higher concentration, they remained unchanged.

The conclusion we draw from these effects is that an excess of agar or gelatin inhibits lysis of bacteria by preventing water from entering and disrupting bacterial cells.

[1] d'Herelle, "Bacteriophage and Its Behavior," Williams and Wilkins, Baltimore, 1926, p. 89.

[2] Bronfenbrenner, J., Muckenfuss, R. S., and Hetler, D. M., *Am. J. Path.*, 1927, iii, 562.

3912

A Simplified Serological Test for Tuberculosis.

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Montank,[1] in 1924, reported before this Society the results of precipitation tests with tricresol on tuberculous serum. Vernes,[2] in 1923, applied his flocculation test to the study of tuberculosis and in 1926 published[3] the results obtained with the use of a resorcine reagent.

Prior to a description of the present test two points should be stressed: first, that it is based on the Vernes[4] principle of a periodic sinusoidal curve of precipitate; second, that it lacks the precision of the Vernes test and is in this way comparable to the Kahn test in its relation to other luetic reactions.

For the test, 0.5 cc. of centrifugalized blood serum, obtained from patient 3 hours after eating, is placed in a small clean tube and an equal quantity of a 1.25% aqueous solution of chemically pure resorcine in a duplicate tube. The contents of the tubes are then mixed rapidly, first pouring the resorcine on the serum. The tube containing the final mixture is securely stoppered and allowed to remain at an approximate temperature of 25° C. for 4 hours, followed by an over-night refrigeration. The following morning the test is read by inspection. According to the volume and character of the precipitate, results are expressed as minus, plus-minus, plus, 2-plus, 3-plus and 4-plus. There is also an atypical result observed in other pathological conditions, with or without concomitant tuberculosis, which is readily recognized by the large flakes of precipitate, different in character from the typical 4-plus reaction. The resorcine solution must be perfectly colorless, but hemolytic or slightly turbid serum may be successfully tested. During a period of 2 years 290 patients have been examined by this technic in conjunction with the more elaborate Vernes test.[5] Normal controls have also been tested as a check against false reactions.

It has been our experience that non-tuberculous sera give no precipitate, and minus and plus-minus readings correspond to Vernes figures below 20. Quiescent tuberculosis, during periods of slight activity, gives plus and 2-plus readings corresponding to Vernes

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figures from 20 to 30. Active tuberculosis gives readings of 3 plus and 4 plus, corresponding to Vernes figures above 30.

This simple method lacks the delicacy of the Vernes technic, but is capable of giving diagnostic information and requires only inexpensive equipment. Owing to its simplicity, too much significance must not be attached to the results in the hands of the inexperienced.

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[2] Vernes, Arthur, *Comp. Rend. Soc. Biol.*, Paris, 1925, xciii, 1425.

[3] Vernes, Arthur, *Études sur la sérologie de la tuberculose.* Fascicule 4. Maloine et Fils, Paris, 1926.

[4] Vernes, Arthur, *Am. Rev. Tuber.*, 1927, iv, 505.

[5] Baylis, Adelaide B., *Am. Rev. Tuber.*, 1927, iv, 500.

3913

Relation of Osmotic Pressure to Availability of Synthetic Media for Streptococci.

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The osmotic pressure of the environment of an organism is believed to be an important factor affecting its metabolism. Dr. Frankel and her coworkers have made the first attempt to apply this consideration to the investigation of synthetic media.[1] These authors kept all their media isotonic with an M/6 NaCl solution. Falk, in his review of the rôle of certain ions in bacterial physiology, expressed the view that "an attempt to keep osmotic pressure constant is undoubtedly a step toward elucidation of the principles underlying the sound use of synthetic media."[2]

The work described in the present paper includes the determination of the osmotic pressures of four series of entirely different media. Media were prepared and their availability for streptococci tested by procedures already described.[3] Osmotic pressures were calculated from the freezing point depressions. The authors were aware, at the outset, of the inaccuracies in this method due to the high molar concentrations of several of the mixtures used. The errors were ignored in order to obtain figures for preliminary comparisons.

The significance of the details obtained may be brought out perhaps most clearly in the following tabulations:

TABLE I.

	Osmotic Pressure in Mm. Hg.		
	12 to 49	53 to 151	666 to 915
Total No. of Media	18	12	16
No. showing growth	16	6	7
Per cent showing growth	89	50	48

TABLE II.

	Osmotic Pressure in Mm. Hg.				
	12-40	40-49	53-151	666-813	817-915
Total No. of Media	10	8	12	8	8
No. showing growth	9	7	6	4	3
Per cent showing growth	90	87.5	50	50	37.5

In all, 46 media were used in this study. When they are divided arbitrarily into three groups on the basis of osmotic pressure, 89% of those with osmotic pressure below 50 mm. Hg. permit viability for shorter or longer periods. Fifty per cent permit viability when pressures are below 151 and above 53 mm. Hg., and 44% of those having osmotic pressures between 666 and 915 mm. Hg. permit viability. (Table I.) Similar gradations appear again when the media are divided into 5 groups. Ninety per cent of the media with pressures below 40 mm. Hg. showed growth, 88% of those having osmotic pressures between 40 and 49 mm. Hg., 50% of those having osmotic pressures above 666 and below 813 mm. Hg. and 38% of the media with osmotic pressures between 817 mm. and 915 mm. Hg. (Table 2.)

Therefore, the number of media which permitted growth increases as the osmotic pressure decreases.

[1] Frankel, F. H., Barber, H., Pyle, E., *J. Infect. Dis.*, 1919, xxiv, 9.

[2] Falk, I. S., *Bacteriological Abstracts*, 1923, vii, 49.

[3] Krasnow, F., Rivkin, H., and Rosenberg, M. L., *J. Bact.*, 1926, xii, 385.

3914

Comparative Studies on Autoplastic Lymph Node and Thymus Transplants.

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(Introduced by David Marine.)

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Studies by numerous investigators on the histogenesis of the thymus gland have failed to clear up the origin and biological significance of the small thymic cell. In order to secure additional data

concerning the origin of these cells, the method of autoplastic thymus transplantation with careful histological studies of the daily regenerative changes which take place in the growing transplants was utilized.[1] It was concluded from these experiments that the small thymic cell in the rat arises from the reticular epithelium.

In an effort to gather further evidence on this much mooted question, the regeneration of a series of lymph node transplants has been studied and compared with transplants of the thymus in rats of the same age, sex and strain. To our knowledge no detailed nor extensive studies are available concerning successful transplantation of lymph nodes. In our studies, young albino rats were used. Pieces of inguinal lymph nodes, approximately 8 mm. in diameter were planted into prepared pockets in the abdominal wall. The presence of infection in these nodes in young rats is infrequent.

At the end of the first 24 hours the lymph node transplants show almost complete destruction of both reticular and lymphocytic elements except for a small zone of well preserved lymphocytes at the periphery, with invasion by polymorphonuclear leucocytes, rapid phagocytosis and marked congestion of the surrounding blood vessels. At 48 hours there is striking evidence of proliferation of the reticular cells from the outer zones, with extension of these elements in finger-like processes toward the center of the transplant. In the outer zone of regenerated reticulum are seen many newly formed lymphocytes. During the third day mitotic figures are numerous. Small lymphocytes which are present as early as 24 hours increase in the outer zone of the transplant. Transitional forms between fully developed lymphocytes and large oval reticular cells can be distinguished. During the following days, regeneration of both lymphocytes and reticular elements continues and the small focus of degeneration which is still present in the center of the plant becomes progressively smaller. About the 6th day, giant cells appear about the center of necrosis within the center of the plant. These giant cells appear to be formed by the fusion of reticular cells. By the 8th day regeneration of the node is almost complete, masses of lymphocytes obscure the underlying reticulum, and in places have formed spherical accumulations resembling the follicles of the normal gland, with beginning formation of germinal centers.

Similar studies on thymus transplants were made. In comparing the regeneration of the thymus to that of the lymph node it is noted that: (1) lymph node transplants regenerate more rapidly than thymus transplants of approximately the same size. (2) In the thymus the entire plant is almost completely replaced by reticulum before any small cells appear; these later occur in scattered

islands throughout. In the lymph node, however, lymphocytes are always present in the peripheral zone and both the lymphocyte and the reticulum regeneration parallel each other. (3) In both types of transplants, the reticulum arising from surviving reticular elements extends in finger-like projections from the periphery of the transplant; the small cell appears to arise from the reticular cells; giant cells appear by fusion of reticular cells about the central area of necrosis in the course of the regeneration.*

* In similar studies on the thyroid and spleen transplants, no giant cell reaction appeared about the central area of necrosis in the regeneration of the transplants.[2-7]

[1] Gottesman, J. Marmorston, and Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 45.

[2] Gottesman, J. Marmorston, and Gottesman, J., unpublished studies.

[3] Manley, O. T., and Marine, David, PROC. SOC. EXP. BIOL. AND MED., 1914, xii, 202.

[4] Manley, O. T., and Marine, David, *J. Am. Med. Assoc.*, 1916, lxvii, 260.

[5] Manley, O. T., and Marine, David, *J. Exp. Med.*, 1917, xxv, 619.

[6] Marine, David, and Manley, O. T., PROC. SOC. EXP. BIOL. AND MED., 1916, xiv, 123.

[7] Marine, David, and Manley, O. T., *J. Exp. Med.*, 1920, xxxii, 113.

3915

Mechanism of Gastric Secretion. The Nature of Gastric Juice of Constant Maximum Acidity.

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In a recent paper,[1] it was reported that, by a modification of the usual technic for collecting gastric juice from a dog's auxiliary stomach pouch, a fluid of constant acidity was obtained. Independently of whether the secretory stimulus was food or histamine, the pH of the pouch juice had a constant value of 0.90±0.01. The difference (a decrease of 0.02 pH) between this and the value originally reported may be due to individual variation as well as to improved technic. With further modifications in the latter, the maximum acidity may be found to be even slightly higher.

Subsequent to the above report, the acid values of some representative samples were determined by titration. The micro-method employed involved titration to a definite end point with the aid of

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comparator tubes of known pH. For total acidity, phenol red at pH 7.8 was used; for free acidity, brom-phenol blue at 3.5. Also, in the case of a few of these samples, total chlorine was determined by the micro-method of Van Slyke.[2] From the data so far obtained the following may be observed:

(1) The average total acidity (.157 N) is higher than any corresponding value hitherto reported. (2) The combined acidity of "Constant pH" juice, determined as the difference between free and total acidities, is negligibly small (.003 N); *i. e.*, this fluid contains practically no protein or other buffer substances. (3) Comparison of the average values for total acidity (.157 N) and for total chlorine (.167 N) indicates a very small difference (.010 N). In fact, considering that it may be impossible to eliminate last traces of the mucus secretion and a serous transudate, this difference may also be within the limits of error of the method. These results imply, therefore, that the HCl solution elaborated by the acid-secreting cells contains very little if any of the metallic chlorides found in the blood and other tissue fluids. (4) Calculation of the freezing point for HCl solutions of .157 N and .167 N concentration, taking $\alpha = .93$, gives values of —0.56° C. and —0.60° C. respectively as limiting values for the gastric juice under consideration—assuming the absence of appreciable quantities of other substances. For mammalian blood, the value of this constant is usually given[3] as about —0.60° C,—thus suggesting the possible isotonicity of this acid secretion.

Work now under way is directed towards a determination of: (a) The actual freezing point depression of gastric juice obtained by this technic; and (b) The presence or absence in this fluid of substances other than HCl.

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[2] Van Slyke, D. D., *J. Biol. Chem.*, 1923, lviii, 523.

[3] Mathews, A. P., *Physiol. Chem.*, 1925, 564.

3916

Production of Placentomata in Rats Injected with Anterior Hypophyseal Fluid.

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Evans and Long[1] have reported that in rats injected with extract of anterior lobe of the hypophysis of beef, oestrus may never occur, or occur only at long intervals. The ovaries of such animals weighed twice as much as in control animals, showing the presence of very abundant lutein tissue and the absence of normal Graafian follicles. Teel[2] was able to prove that this lutein tissue is functional as regards its ability to sensitize the uterine mucous membrane, as determined by the placentoma reaction of L. Loeb.[3] The uterine mucous membrane of injected animals exhibits the decidual cell reaction 5 or 6 days after the beginning of the treatment.

However, this typical reaction can be obtained only during a short period of the life of the corpora lutea, as shown by the following experiments:

Group I. Female rats, which were having normal cycles, were injected daily with anterior hypophyseal fluid. On the day of the fifth injection, a loop of silk thread was inserted through the lumen of the uterus to produce an injury to the uterine mucosa. Between the fourth and the seventh days after operation, the animals were killed. No cycle occurred during the experiments. In every animal large placentomata were found in the injured uterine horn, not exclusively limited to the sites of injury, but including in some cases almost the total length of the horn. The ovaries contained in each case numerous corpora lutea and were larger than in a normal animal. Some of these ovaries exhibited the typical aspect described by Evans and Long as "mulberry ovaries." This first group of experiments confirm the results of Evans and Long and of Teel.

Group II. Loops of silk thread were inserted through the lumen of the uterus of female rats at different periods of the oestrous cycle. Daily injections of anterior hypophyseal fluid were made, starting on the day following operation, and the animals were sacrificed after 5 to 12 injections. In some animals a very slight tumefaction was found at the sites of injury; other animals did not exhibit any reaction. In no cases were typical large placentomata obtained. However, the oestrous cycle was stopped during injections and the

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ovaries contained large and numerous corpora lutea. These experiments seem to indicate that large placentomata can be obtained only after the uterine mucosa has been sensitized by the action of the corpora lutea.

Group III. Female rats were injected daily with hypophyseal fluid. On the day of the 10th injection, loops of thread were inserted, and the animals received 5 daily injections after the operation. In no case was the placentomata reaction observed. The same negative results were obtained in animals injected daily, injured by loops of thread on the 23rd to the 27th day of treatment and injected for 5 to 18 days after operation. In this group also, the oestrous cycle was inhibited during the experiments; the ovaries contained numerous corpora lutea and some were typically "mulberry ovaries."

These experiments can be explained by the experiments of Hisaw,[4, 5] and Weichert.[6] The first author showed that corpus luteum extract produces a relaxation of the pubic symphysis of the castrated guinea pig, only when the animal is under the influence of the follicular hormone. Injecting the same extract, Weichert was able to produce placentomata in spayed rats, but only after the animal was put in artificial oestrus by follicular hormone.

It seems that the reason it is not possible to obtain the placentomata in animals injected with hypophyseal extract and presenting persistent corpora lutea for 10 days or more is because they are too far away from the last oestrum and consequently not under the influence of the follicular hormone. In such conditions the uterine mucosa can not react to a stimulus by producing decidual cells.

Conclusions. 1. In animals injected daily with anterior hypophyseal fluid, and presenting numerous persistent corpora lutea, it is possible to obtain regularly large placentomata if the injury of the uterine mucosa is produced around the fifth day of injections and the animal killed 5 to 7 days after operation.

2. If the injury of the uterine mucosa is made the day before the injections started, it is not possible to obtain large placentomata. A slight enlargement at the sites of injury may be observed.

3. If the injury of the uterine mucosa is made after 10 days or more, placentomata are never obtained.

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3917

Production of Placentomata in Normal and Ovariectomized Guinea Pigs and Albino Rats.

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The work of Loeb[1] in producing placentomata in the normal guinea pig by irritating the uterine mucosa between the fourth and ninth days after oestrum, suggested a comparable experiment in the albino rat. Negative results were obtained in the normal rat and were perhaps due to the oestrous cycle being so short that insufficient time elapses for a proliferation of decidual cells before the next cycle occurs. With the discovery that potent extracts of the corpus luteum could be prepared, as described by Hisaw[2] the problem was attacked from a different angle. The corpus luteum hormone when injected into the rat caused an inhibition of ovulation. This hormone was injected into rats for 2 days following oestrum, at which time the uterine mucosa was stimulated and the injections continued for 4 days. The rats were killed at this time and placentomata were found.

Ovariectomized rats were treated with corpus luteum hormone in the same manner and no placentomata were formed. If, however, such rats were first brought into oestrum artificially by injections of the follicular hormone and then treated as above, placentomata were formed. Apparently the follicular hormone is necessary to put the uterus in a proper physiological condition before it will respond to the corpus luteum hormone. Moreover in ovariectomized rats which have been injected first with follicular hormone followed by the corpus luteum hormone, stimulation of the uterine mucosa at any time during the interval between artificial oestrum and the fourth day after, produced these effects, while the normal rat is unresponsive until the third or fourth day after oestrum. Experiments are now being carried on to determine the length of time which may elapse before this physiological condition produced by the follicular hormone disappears so that placentomata can not be formed even though the corpus luteum hormone is present.

In guinea pigs, which on the fourth day after oestrum were ovariectomized and in which the uterine mucosa was stimulated, no placentomata were noted when the animals were killed 4 days later. If, however, the corpus luteum hormone were administered at the time of operation and continued for 4 days, then placentomata were

formed. Attempts were made to produce placentomata in the guinea pig between the ninth and fourteenth days after oestrum, a period during which they cannot normally be produced in the animal. These animals were injected daily with the corpus luteum hormone from the sixth to the fourteenth day. The mucosa was stimulated on the tenth day but no placentomata were formed. It would seem as though too long an interval had elapsed since the previous oestrum, and the effect of the follicular hormone had been lost. This, too, is being investigated at the present time.

These results are in accord with the findings of Brouha,[3] who by injecting extracts of the anterior lobe of the hypophysis into the rat causes a great enlargement of the corpus luteum and the inhibition of ovulation over extended periods. He finds that in such rats placentomata may be produced only between the fourth and seventh days after ovulation, although the corpora lutea persist without any indication of involuntary changes having taken place. The probable explanation of this is that the follicular hormone is absent after the seventh day so that the uterus is not in the proper physiological condition to respond. Hisaw, by experiments in which the pelvic ligaments of the guinea pig were relaxed by injections of corpus luteum extracts, finds that relaxation occurs only when the animal is under the influence of the follicular hormone. This influence is manifested from the fourth day before oestrum until 7 days after, the intensity reaching a maximum at oestrum and gradually diminishing until practically all effect is lost after the seventh day. The reaction in this case occurs under the same conditions as those found necessary for the production of placentomata.

[1] Loeb, Leo, *J. Am. Med. Assn.*, 1908, l, 1897.

[2] Hisaw, F. L., reported at the meeting of the A. A. A. S. at Nashville, Tenn., 1927.

[3] Brouha, L. L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 488.

3918

Erythrocytophagic Capacity of the Hepatic Peritoneum in the Splenectomized Horned Toad, Phrynosoma Solare.

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The data are a byproduct of splenectomy experiments. From an operative standpoint the horned toad seemed the most favorable

reptilian material. However, both controls and experimentals succumbed indifferently, until at 74 days after operation only 1 of each

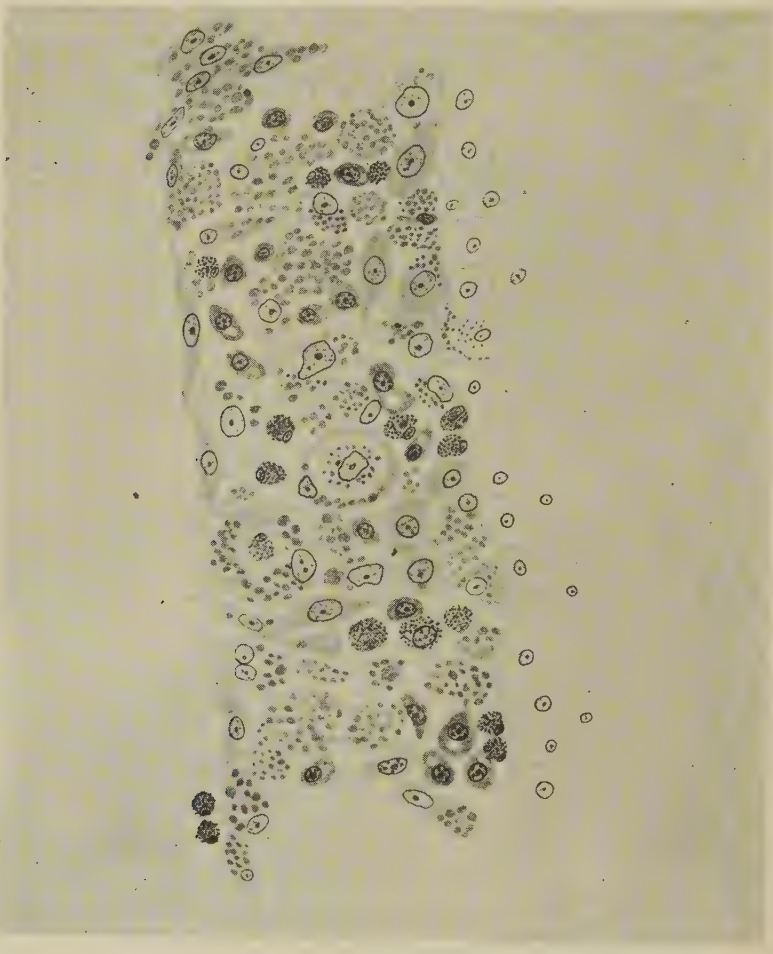

FIG. 1.

Portion of the erythrocytographic hepatic peritoneum of a splenectomized female. On the left surface appear three cells of intact mesothelium. At the upper left hand border the mesothelial cells have assumed columnar shapes. At the lower left hand border occur two eosinophils over a denuded area. Along the right hand border is indicated in outline the hepatic parenchyma. One of these cells, near the middle, is shown with a considerable content of ingested hemoglobin debris. The hyperplastic erythrocytophagic capsule consists predominantly of large pale mononuclear cells whose cytoplasm contains many spheroidal red cell fragments. Many of these macrophages are in process of disruption. The small cells with black granules represent eosinophils. The lymphocyte-like cells, with conspicuous cytoplasmic vacuole are prospective, third generation, macrophages, *in situ* derivatives of stromal cells. Helly fixation, Giemsa stain. X600.

remained alive. The splenectomized toad was a large female, apparently in perfect health at the time it was killed and tissues preserved for microscopical examination. Normally the spleen represents practically the sole organ of blood formation. No sign of splenic regeneration, or compensatory myeloid metaplasia in any of the tissues examined could be detected. But sections of the liver showed a striking change in the capsule. Both serosal cells and subserous fibroblasts had assumed quite unexpectedly intense phagocytic properties (Fig. 1).

The hyperplastic and extensively phagocytic capsule has acquired an average thickness of approximately 1 mm. The normal capsule includes barely more than a mesothelial layer. In an effort to explain this unusual condition it was recalled that at operation this animal bled profusely. The splenectomy experiment, done for the purpose of studying the hemocytopoietic effect, had accordingly changed to one concerning the reaction of the peritoneum to whole blood in the cavity, in the absence of a spleen. The intestinal peritoneum showed no phagocytic activity, the ovarian tissues a moderate amount, the hepatic peritoneum a very intense degree.

The method by which the fragmenting erythrocytes are handled by the cells of the hepatic peritoneum is especially interesting. The irritation resulting from the presence of blood in the peritoneal cavity stimulated the mobilization of mononuclear and eosinophilic leucocytes in the cavity. The mononuclears ingested the hemoglobiniferous and nuclear fragments, apparently selectively, and carried them to the hepatic capsule. Here they suffered disruption and deposited the debris in the interstitial spaces. The presence of these relatively large blood-cell fragments stimulated hypertrophy and phagocytic activity on the part of both the serosal cells and the underlying connective tissue cells. Apparently also, both serosal cells and subserous macrophages (clasmatocytes) migrate from the hepatic capsule into the peritoneal cavity and return laden with debris. These cells likewise disrupt and deposit their ingested contents, which are reingested and converted into smaller particles by fibroblasts transformed into macrophages. Subsequently this second generation of macrophages suffers dissolution with deposit of contents in the intercellular spaces.

These masses of smaller hemoglobin derivatives (hemosiderin) stimulate the origin of a third generation of macrophages. At the stage examined these cells occur extensively, both singly and in small compact groups. These cells are much smaller, sharply contoured deeply staining elements. They originate *in situ* and have

the general nuclear and cytoplasmic characteristics of small lymphocytes, except that the cytoplasm is more extensive and invariably contains a lighter staining circular area close to the nucleus. These cells represent fibroblast differentiation products. A few are in mitosis. They are prospective macrophages. A few contain 1 or 2 fragments of hemoglobin debris. A final stage, sparsely represented, appears to be one in which these cells with their ingested hemoglobin debris in the shape of minute dark brown granules have suffered disruption in the intercellular spaces of the superficial parenchymal hepatic cells. Here the granules are finally ingested by the liver cells to be converted into bilirubin.

Three additional striking details concern the apparently functional specificity among the subserous macrophages, these not ingesting both nuclear and cytoplasmic fragments at the same time but only one or the other variety; the presence of a few phagocytic eosinophils with hemoglobiniferous fragments; the presence of a few multinucleated giant cells; and the occurrence of a number of "cannibal" macrophages which have ingested one or several older macrophages and occasionally one or several eosinophils. Apparently the phagocytic capacity of peritoneum is determined at least in part by its location with respect to tissue that can dispose most advantageously of the phagocytosed materials.

3919

Experimental Scurvy in the Guinea Pig.

A. W. MEYER AND L. M. MC CORMICK.

*From the Department of Anatomy, Stanford University.**

Increasing abstinence and loss of weight, decreasing activity and drowsiness, or marked nervousness and greatly heightened activity in some animals, are characteristic of early scurvy. In later stages, stiffness, weakness and even complete helplessness may supervene in the hind quarters, especially of young animals. Many animals were very fat at time of death. Gross hematuria and melena, drooling and a foul odor were sometimes present.

Evidences of pain were noticed seldom, and bleeding and ulcerated gums, loose incisors, fever and constipation were never observed. Thirst and chewing movements, even when complete anor-

* This investigation was generously assisted by grants from the Committee on Researeh of the American Medical Association.

exia seemed present, were noted in most animals, and a lowered temperature in all of them. Not infrequently a relatively large mass of soft, foul feces protruded from the anus in the late stages. and a foul odor seemed to emanate from the mouth. Swollen joints were seen only in very young pigs.

Recovery from the scorbutic condition was often possible in young animals, which had suffered an apparent paralysis of the hind quarters, as late as approximately 6 hours before death, but these pigs always had permanently rigid hind legs because of lack of mobility at the knee. Ankylosis at the knee was never encountered. General edema was encountered only once in a large series of animals, but some edema was relatively common in the peri-vesical fat.

At necropsy the well-known subcutaneous and deep hemorrhages, even into the central and peripheral nervous systems, were present and grossly pseudo-pneumonic areas in the lungs occurred in most animals. Next to these changes, the most conspicuous change was the occurrence of fatty degeneration of the liver, even up to pure whiteness at the caudal margins. Fatty infiltration and degeneration were also found in the kidneys, adrenals, lungs, pancrease and, to some extent in some animals, in the skeletal muscles, and in one case it was present in a very marked degree in the walls of the pancreatic artery.

The intestine often was totally unresponsive to pinching and the stomach sometimes was greatly distended with gas. The molar teeth were invariably loose beginning with the last, and the implanted portions were reduced in length and caliber. Hemorrhages were present in the pulp of the incisors also, but there was no evident change in the physical properties of any of the teeth.

Vacuolation and fenestration were present in many organs, including the bone marrow and the central nervous system, and pronounced hydropic degeneration was present in the skeletal, the gastro-intestinal and the arterial musculatures.

Desquamation and disintegration of epithelia was quite common in the bronchial and intestinal tracts, in the kidneys and urinary bladder and in the biliary and pancreatic ducts. Complete disintegration of epithelia was observed also in the liver, kidney, adrenal and testis, and plaques of liver cells were found in some large hepatic veins. Plaques of epithelium from the convoluted tubules were present in the collecting tubules, and degenerated germinal epithelium in the *ductus deferentes*. Degenerative changes also were found in the interstitial cells of the testes, in those of the pancreatic islands and in nerve and ganglion cells and nerve fibers.

Proliferative changes were encountered only in the costal cartilages and bone marrow and the predominant picture was one of destruction. The walls of small vessels were found completely disintegrated and those of larger vessels frequently were entirely destroyed locally, and although the presence of intravascular hemolysis was not established, the histologic study of tissues from scorbutic pigs gave the impression that a lytic process almost universal in extent was present, and that the hemorrhages were due to destruction of the walls of blood vessels through lysis.

Marked fetal scurvy was also obtained and presented all the characteristics of scurvy in the mature guinea pig.

In a study of the blood, McCormick found a decrease in the number of erythrocytes, hemoglobin and color index, and an apparent decrease in fragility, a relative decrease in the number of lymphocytes and an absolute increase in the polymorphonuclears. There also was an increase in the reticulated and nucleated blood cells and leucocytes. A large series of counts on normal animals, as well as a first count on the experimental animals, served as a basis for comparison. None of the scorbutic animals had a rise in temperature, as determined by rectal measurements.

3920

Permeability of the Upper Respiratory Mucous Membrane for Bacteria and Their Products.

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Observations made in this laboratory during the past year or two have disclosed the fact that, in the case of the rabbit at least, bacteria and their products readily and invariably pass through the mucous membrane of the upper air passages when in contact with or growing upon its outer surface.

When virulent pneumococci are put into the nares they soon appear in the blood stream and set up a rapidly fatal infection. Less virulent organisms also find their way into general circulation but the resulting infection is frequently overcome and the animal recovers. If killed pneumococci are put into the nares the organisms themselves cannot be demonstrated in the blood but antibodies speci-

fic for them soon appear in the plasma, indicating that either the cocci or their products penetrated the mucous membrane. It is possible, of course, that the antibodies may be produced locally and then absorbed.

Similar results can be obtained with *Bact. lepisepticum*—an organism natural to the host. The less virulent strains of this species often occur in the upper air passages of rabbits in the carrier state, but more virulent strains may produce local or general infections. The occurrence of these organisms in the upper air passages under any condition is followed by the appearance of antibodies in the blood serum. Here also a systemic immunity may be produced by putting killed cultures into the nares.

An observation along the same line has been made with *B. bronchisepticus*. This organism occurs very generally in the upper air passages of rabbits, chiefly in the carrier state. Apparently every rabbit exposed to *B. bronchisepticus* becomes a carrier, but rabbits raised under special conditions can be kept free of it. The serum of such rabbits does not contain any antibodies for this organism. This provides an excellent opportunity for determining the systemic effect of the carrier state. Samples of serum were collected from a number of rabbits which had never come in contact with *B. bronchisepticus*. The rabbits were then inoculated intranasally with a small amount of culture. Serum was collected on the sixth and eighth days following inoculation. The 3 sets of sera were tested for specific complement-fixation. All of the samples collected before inoculation were negative in a dilution of 1-4. The 6-day samples were all positive in dilutions varying from 1-8 to 1-32 and some of the 8-day samples were positive up to a dilution of 1-512. It is not believed that such rapid immunization would follow subcutaneous inoculation.

Summary. Virulent bacteria put in the nares of rabbits soon appear in the general circulation and produce septicemic infections. Less virulent bacteria also appear in the blood but may not cause infection. Killed bacteria put into the nares stimulate systemic antibody production. Bacteria occurring in the nasal passages in the carrier state stimulate a very rapid antibody response. These observations indicate that the mucous membrane of the upper air passages is readily permeated by bacteria and their products.

3921

Effect of Prenatal and Postnatal Injections of the Pituitary Gland in the White Rat.

E. L. COREY. (Introduced by J. S. Nicholas.)

From the Osborn Zoological Laboratory, Yale University.

The present study was undertaken in an attempt to ascertain the time at which the pituitary hormone begins to act upon the organism. Smith and Engle[1] have shown that transplantation of portions of the pars anterior of the pituitary into the young rat causes marked responses in the genital system attributable to the influence of the grafts upon the gonad, which in turn modifies the entire genital tract by means of its enhanced secretions. The gonad has, therefore, been selected as an organ in which there is a definite response to the pituitary hormone which might be used as an index of the first activity of the physiological effect of that secretion.

For the technique employed in performing laparotomy upon the pregnant rat preparatory to the injection of foetuses see Nicholas.[2] Previous failures of many workers to obtain positive results with injections of the gland, single transplants, and pituitary feeding renders the success of the method employed of some interest. The entire pituitary gland was excised from a freshly killed male or female adult rat and macerated under aseptic conditions in a ground-glass mortar in 1 cc. of 0.9% sodium chloride solution. The resultant fluid was used for injection, the same concentration as above being constantly maintained. In all prenatal injections a 27 gauge needle was used, and from .02 to .05 cc. were administered intraperitoneally at each injection. In postnatal treatment from 0.1 to 0.25 cc. were given, either intraperitoneally or subcutaneously, the type of injection being noted in the protocol. Prenatal injections were made upon alternate days; after birth, daily.

The youngest animals injected were those in the fifteenth day of

INJECTED ANIMALS.

Age (days)		No. litters	No. individuals
15	Prenatal	5	26
16		2	13
17		2	16
18		3	19
New-born		2	26

Four litters supplied the control animals.

foetal life. Sixteen, 17, and 18-day embryos were similarly injected. Gonads of both sexes were preserved for sectioning at various stages of both injected and non-injected animals. All material was fixed in Bouin's solution.

Prenatal injection in no way hastened the differentiation in the gonad. It is thus evident that the gonad of the rat foetus is incapable of response to the pituitary hormone. No evidence of sexual maturity was observed in either sex until the tenth day of postnatal life, when the effect was most pronounced in the male. This effect evinced itself in an apparent increase in tubule length as well as by a considerable thickening of the tubular wall. There is an undoubted increase in the size of the interstitial cells, and the tissue presents a dense and closely-packed appearance. We attribute this close-packed appearance to the increase in length and diameter of the tubules. No reconstructions have as yet been made to render this point a certainty.

In the ovary the earliest advance in maturity of the injected animals over the controls appeared at approximately the fifteenth day. No corpora lutea were present but the follicles are larger in diameter and more nearly approaching maturity than in the normal animal if judged by the amount of follicular fluid present. At the twentieth day this precocious development is more marked. In no case were corpora lutea observed nor could eggs be discerned within the lumen of the sectioned tube.

On the basis of the above results, the physiological effect of the pituitary hormone upon the gonad first shows itself in *both* sexes between the tenth and fifteenth days of postnatal life.

[1] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, xl, 159.

[2] Nicholas, J. S., *Anat. Rec.*, 1925, xxxi, 385.

3922

Studies on Quinine and Quinidine V. Do They Have a Specific Action on Autonomic Nerve Ends?

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In an earlier paper in this series[1] it was shown that epinephrine loses part or all of its pressor activity in dogs following injections of quinine or quinidine because of a partial or complete paralysis of

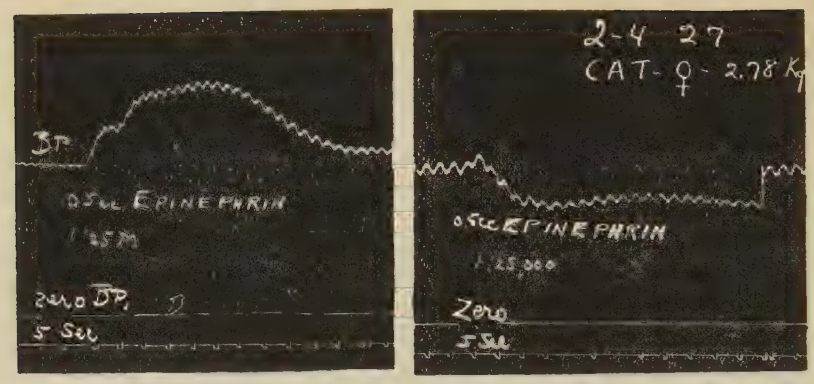

FIG. 1.

Vasomotor reversal in the cat. Between the two injections of epinephrine, 12 mg. Quinidine hydrochloride were injected. Morphine-urethane anesthesia.

the peripheral vasomotor mechanism. This was localized as being on the endings because pituitary solution was still effective in raising the blood pressure. The work has been extended to cats, and it has been found that there is not only a loss of pressor activity but also in some cases a very clear cut vasomotor reversal. Figure 1 illustrates such a case. Here approximately 3 mg. of quinidine per kilo have completely reversed the action of epinephrine. In other similar experiments this same result has been obtained not only from the injection of epinephrine, but also from stimulation of the splanchnics. These findings in cats and dogs suggest a similarity in action between quinine (or quinidine) and the ergot alkaloids. When the reaction of the rabbit uterus to epinephrine before and after quinine or quinidine was examined, there was again noted this reversal. This phenomenon has also been noted by Stake[2] and Langecker.[3] The isolated uterus of the virgin cat which relaxes to epinephrine, gave a greater degree of relaxation after administration of quinine. In 2 experiments the stimulant action of epinephrine for the retractor penis of the dog has been reversed. Because of these positive findings the experiments have been further extended to determine whether quinine and quinidine could be said to act specifically on the motor divisions of the sympathetics. The experiments have been uniformly negative. It has not been possible to show any antagonism for the action of epinephrine or the cervical sympathetic on the dilator iridis or the orbital smooth muscle (cats, rabbits). Neither the sympathetic secretion (cats, dogs) nor the chorda secretion (dogs) from the submaxillary gland is altered in amount by quinine. The augmentor effect of epinephrine on the heart persists after these alkaloids. And finally epinephrine pro-

duces its characteristic rise in blood sugar after quinine though this has been shown to be prevented easily by ergotoxin or ergotamine. When quinine bisulphate, 20 mg. per kilogram is given intravenously to fasting rabbits, there is a rise in blood sugar, the maximum value rarely being as high as 200 mg. per 100 cc. This maximum is reached in from 60 to 90 minutes, after which it gradually falls. Epinephrine, 0.2 cc. of 1:10,000, injected through a period of 10 minutes, intravenously, at the time of maximum quinine effect, produces a second increase, which may go above 300 mg. If quinine has any antagonism for this effect of epinephrine, it is very slight.

Because of these negative findings, no further antagonisms have been studied, for though others may be found there are sufficient exceptions to make impossible the generalization suggested earlier.[4] Quinine and quinidine, though apparently having a selective action on the motor sympathetics to the blood vessels in cats and dogs, do not have such an action for all the motor fibers of the sympathetic division.

[1] Nelson, E. E., *Arch. intern. de Pharmacodyn. et de Therap.*, 1927, xxxiii, 197.

[2] Stake, T., *Compt. Rend. Soc. Biol.*, 1926, xciv, 954.

[3] Langecker, H., *Arch. exp. Path. u. Pharmakol.*, 1926, cxviii, 49.

[4] Nelson, E. E., *J. Pharm. Exp. Therap.*, 1927, xxxi, 209.

Missouri Branch.

St. Louis University Medical School, February 29, 1928.

3923

Observations on Urobilinogen in Urine.

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From the St. Louis University School of Medicine.

Urobilinogen of urine was quantitated by the method of Terwen.[1] As would be expected from the work of McMaster and Elman,[2] definite increases in urinary urobilinogen were found in cases of liver pathology. Increased values were also found in some cases of diabetes mellitus, most of which showed some suggestive evidence of an accompanying liver pathology. This was proven in 2 cases coming to autopsy, one of which showed cirrhosis and the other marked passive congestion of the liver.

Increases were also found in some cases in which definite evidence of liver pathology could not be found. Active cases of pernicious anemia showed this finding. Cases of severe nephritis frequently showed increased urinary urobilinogen. It is questionable whether this should be attributed to a possible accompanying liver lesion or to increased renal permeability to this substance. Nephritic cases with a high urobilinogen output are usually accompanied by anemia. A similar high urinary pigment output is noted in other secondary anemias of obscure etiology. This suggests that loss of pigment may play some rôle in the production of the anemia. A few cases of renal glycosuria were found to have high values for urinary urobilinogen. This may also be due to increased renal permeability.

The occurrence of increased output of urobilinogen in urine of cases in which no definite evidence of liver pathology could be found, renders difficult the use of this reaction as a test of liver function.

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[1] Terwen, A. J. L., *Deutsch. Archiv. f. Klin. Med.*, 1925, cxlix, 72.

[2] McMaster, P. D., and Elman, R., *J. Exp. Med.*, 1925, xlii, 99.

3924

Cyclic Changes in Vaginal Flora of the Rat.

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The flora of the vaginal canal of the normal rat has been studied in relation to the oestrous cycle. Cultures were made upon blood agar plates and the number of organisms and the types of organisms were determined. At the same time the stages of the oestrous cycle were noted by means of stained smears of the vaginal secretions. We have also studied the effect of spaying rats and the influence of administration of ovarian extract to such spayed rats.

It appears that in the normal dioestrous stage certain gram negative bacilli are the principal, if not sole, organisms found in the vagina of the rat. This is true in both the mature and immature virgin, and it appears from a single observation that during the early stages of pregnancy these same organisms are the predominant organisms in the vaginal canal of the pregnant rat. These same organisms predominate in the vaginal canal during oestrum, proestrum and metaoestrum, but during oestrum there appear in addition large numbers of Gram positive cocci, a few Gram negative cocci, and also a few Gram positive bacilli. This cyclic change in the bacterial flora of the vaginal canal is quite as constant as are the cytological changes which serve to mark the various stages of the oestrous cycle.

When a rat is spayed the cyclic bacteriological changes cease, just as do the cytological changes. If such a spayed rat receives an injection of ovarian hormone, the bacteriological findings become essentially those of the oestrous stage, just as the cytological picture changes to that of the oestrum.

It is evident then that the changes of the bacteriological flora of the rats' vagina are in some manner intimately related to ovarian activity. The exact relationship remains to be worked out.

3925

An Acoustic Probe.

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The writers first described the bone activating telephone receiver in this journal.[1] The apparatus was used in studying the problem of the minimum audition for bone transmitted sound in normal and pathological cases. The results of these researches have appeared from time to time in the *Annals of Otology,* the *Laryngoscope* and the *Archives of Octololaryngology.* Attention has already been attracted in various papers to the fact that not until the character of the physical disability in the sound transmission apparatus has been established may we hope to arrive at a proper operative solution for the amelioration of some types of deafness. The acoustic probe is nothing more than a miniature bone activating receiver provided with a long slender stalk which is to be used as a probe. When the appliance is hooked up with an electric adiometer the minimum acuity for bone transmitted sound may be determined when the tip of the probe is applied to the handle of the malleus, the long process of the incus and the stapes. This necessarily must be done with reflected drum membrane and under light local anesthesia. It is hoped that in selected cases this method of "feeling one's way" along the sound transmission system will show where a blocking of the drum membrane vibration takes place. Once a mechanical lesion is determined and located, operative correction of the disability may be anticipated. The probe is also to be employed in determining the location of the "hot spot" found in individuals who in the absence of drum membrane and outer ossicles find the cotton plug prothesis of great benefit to air acuity.

[1] Pohlman, A. G., and Kranz, F. W., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 335.